A STUDY ON BLOOD STREAM INFECTIONS IN HIV PATIENTS IN A TERTIARY CARE HOSPITAL

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DECLARATION

I declare that the dissertation entitled **"A STUDY ON BLOOD STREAM INFECTIONS IN HIV PATIENTS IN A TERTIARY CARE HOSPITAL"** *submitted by me for the degree of M.D. is the record work carried out by me during the period of* ***May 2009 to May 2010*** *under the guidance of Professor* ***Dr. S. GEETHALAKSHMI M.D., Ph.D.,*** *Professor of Microbiology, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Microbiology (Branch IV) examination to be held in April 2011.*

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CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON BLOOD STREAM INFECTIONS IN HIV PATIENTS IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **DR. S. AMUTHAVENI,** during the period of her Post graduate study from June 2008 to April 2011 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Government General Hospital, Chennai-600003, in partial fulfillment of the requirement for **M.D. MICROBIOLOGY** degree Examination of The Tamilnadu Dr. M.G.R. Medical University to be held in April 2011.

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CONTENTS

INTRODUCTION

HIV / AIDS is becoming a major threat to the human population across the Globe. According to UNAIDS report, globally there were an estimated 33 million people living with HIV in 2008. There were about 2.7 million new HIV infections and about 2 million AIDS related deaths in 2008[2]. The rate of new HIV infections has fallen in several countries, but has increased in developing countries. Provisional estimates show that there are 22.7 lakh people living with HIV/AIDS in India by the end of 2008 with an estimated adult HIV prevalence of 0.29 percent[47]. Patient infected with HIV suffer from various opportunistic infections, as a result of profound impairment of cellular immunity by the virus. The spectrum of opportunistic infections in HIV-infected patients is related to the microflora of the local environment[25].

Bloodstream infections (BSI) are a major cause of morbidity and mortality among patients infected with HIV[23,64] and is often associated with a poor prognosis[22,33,39,51,57,60,61] , responsible for the immediate cause of death in up to 32% of cases, especially under particular conditions (e.g. intravenous drug abuse, use of a central venous catheter, neutropenia, and a low CD4 T-cell count)[60] . Inappropriate empirical therapy and the presence of other immunosuppressive factors were independent risk factors for

mortality. Ceftriaxone could be used as the initial empiric therapy for HIV-infected patients with suspected CA BSI[50].

Patients with AIDS have the greatest diversity of pathogens recovered from blood, including mycobacterial species, *Bartonella henslae*, *Corynebacterium jeikeium*, *Shigella flexneri*, unusual *Salmonella* species, *Histoplasma capsulatum, Cryptococcus neoformans,* and cytomegalovirus. As is typically observed in other hospitalized patients, organisms such as gram positive aerobic bacteria (e.g., *Staphylococcus aureus, Enterococcus)* and gram negative aerobic bacteria (e.g., Enterobacteriaceae, *Pseudomonas aeruginosa*) are common causes of bloodstream infections in immunocompromised patients[8].

Only a few studies regarding the prevalence of blood stream infections in HIV infected patients are available from South India at present. I took up this study in our institution, to evaluate the prevalence of such infections in HIV patients in our setup in relation to their CD4 T lymphocyte counts and to ascertain the importance of blood culture examination for the detection of blood stream infections that aids in treatment, thereby decreasing the morbidity and mortality due to community acquired and nosocomial blood stream infections.

AIMS OF THE STUDY

1. To determine the prevalence of non mycobacterial blood stream infection in HIV positive and AIDS patients.
2. To establish the causative organisms using the blood culture methods.
3. To determine the antimicrobial susceptibility pattern of the isolates.
4. To determine the risk factors, outcome and influence of long term prophylactic antibiotic treatment.
5. To study the correlation between CD4 and the prevalence of blood stream infections.

REVIEW OF LITERATURE

HUMAN IMMUNODEFICIENCY VIRUS

AIDS was first recognized in the summer of 1981, when the US centers of disease control and prevention reported the unexplained occurrence of *Pneumocystis jirovecii* among homosexual men in Los Angeles and an outbreak of a rare form of cancer among gay men in New York and California, medically known as Kaposi sarcoma. Within months, the disease became recognized in male and female drug users and soon thereafter in recipients of blood transfusions and in hemophiliacs. As the epidemiologic pattern of the disease unfolded, it became clear that a microbe transmissible by sexual contact and blood or blood products or from mother to infants was the most likely etiologic agent.

In 1983, Luc Montagnier isolated the virus from a patient with lymphadenopathy and by 1984 it was demonstrated clearly to be the causative agent of AIDS. In 1985, a sensitive enzyme linked immunosorbant assay was developed[28].

The etiologic agent of AIDS is HIV, which belongs to the family of human retroviruses and the subfamily of lentiviruses. There are two distinct types of human AIDS viruses namely HIV-1 and HIV-2. The two types are distinguished on the basis of genome organization and phylogenetic relationship with other primate lentiviruses[29].

The most common cause of HIV disease throughout the world is HIV-1. HIV-2 was first identified in 1986 in West African patients and was originally confined to West Africa. However a number of cases have been identified through out the world[28].

HIV-1 is subdivided into 3 genetic groups designated M (Major), O (Outlier) and N (Non-M, Non-O) based on sequence diversity within the HIV-1 gag and env genes. HIV-1 group is divided into 9 sub-types (A-D, F-H, and J&K)[10]. Subtypes differ in mode of transmission, geographical distribution and biological characteristics. Subtype C viruses are the most common worldwide. The predominant subtype in Europe and the America is Subtype B. Subtype C is prevalent in India[48].

HIV-1 and HIV-2 have the same mode of transmission. The most common mode of HIV infection is sexual transmission – both homosexual and heterosexual contact. Infection may also occur through inoculation of infected blood products, via transfusion of blood products, transplantation of infected tissues or through contaminated needle[10]. Large multi institutional studies have indicated the risk of transmission following skin puncture with needle contaminated with blood from a HIV infected person as 0.3% and after mucous exposure it is 0.09%. The other mode of transmission is from the infected mother to the child either intrapartum, perinatally or via breast milk[28].

The hallmark of HIV disease is a profound immunodeficiency resulting primarily from a progressive quantitative and qualitative deficiency of the subset of T lymphocytes referred to as helper T cells or inducer T cells. This subset of T cells is defined phenotypically by the presence on its surface of the CD4 molecule which serves as the primary receptor for HIV. HIV uses two major co-receptors namely CCR5 and CXCR4 for fusion and entry[28].

After attachment, HIV RNA and enzymes are released into the host cell. Viral replication requires reverse transcriptase (an RNA dependent DNA polymerase) associated with HIV RNA, producing proviral DNA; this copying mechanism is prone to errors, resulting in frequent mutations. These mutations facilitate the generation of HIV that can resist control by the host's immune system and by antiretroviral drugs. Proviral DNA enters the host cell's nucleus and is integrated into the host DNA in a process that involves HIV integrase. With each cell division, the integrated proviral DNA is duplicated along with the host DNA. Proviral HIV DNA is transcribed to viral RNA and translated to HIV proteins, including the envelope glycoprotein 40 and 120. The HIV proteins are assembled into HIV virions at the inner cell membrane and budded from the cell surface; each host cell may produce thousands of virions. After budding, protease, another HIV

enzyme, cleaves viral proteins, converting the immature virion into a mature, infectious form.

The main consequence of HIV infection is damage to the immune system, specifically loss of CD4+ lymphocytes, which are involved in cell-mediated and, to a lesser extent, humoral immunity. CD4+ lymphocyte depletion may result from the following:

1. Direct cytotoxic effects of HIV replication
2. Cell-mediated immune cytotoxicity
3. Thymic damage that impairs lymphocyte production

Infected CD4+ lymphocytes have a half-life of about 2 days, which is much shorter than that of uninfected CD4+ cells. Rates of CD4+ lymphocyte destruction correlate with plasma HIV level. Typically, during the initial or primary infection, HIV levels are highest ($>10^6$ copies/mL), and the CD4 count drops rapidly. The normal CD4 count is about 750/μL, and the immunity is minimally affected if the count is > 350/μL. If the count drops below about 200/μL, a variety of opportunistic pathogens may produce clinical disease. The humoral immune system is also affected[3] .

LABORATORY INVESTIGATIONS FOR HIV INFECTION

1. Laboratory methods for the diagnosis of HIV infection
2. Laboratory methods for monitoring Stage and Progression of Infection.

Laboratory methods for the diagnosis of HIV infection

1. Indirect Methods (Antibody detection methods)

2. Direct Methods.

DETECTION OF SPECIFIC ANTIBODIES

Detection of anti-HIV antibodies is the main stay of diagnosing HIV. Tests to detect specific antibodies can be classified into

1. Screening tests
2. Conventional ELISA
3. Rapid tests

Rapid tests are in vitro qualitative tests for the detection of antibodies to HIV-1 and HIV-2 in serum, plasma, whole blood, saliva and urine.

- Immunoconcentration
- Particle agglutination
- Immunochromatography
- Immunocomb

Supplemental tests (Confirmatory tests)

- Western Blot
- Immunoblot
- Lineimmuno assay
- Indirect fluorescent antibody test
- Radioimmunoprecipitation tests

DIRECT DETECTION OF HIV INFECTION

HIV Infection is diagnosed mainly by detecting the HIV antibodies. But there are situations where the serology is negative although there is definite evidence of exposure to HIV infection. Direct detection methods are needed in the following settings:

4. To determine HIV status during the window period
5. In health care workers, following accidental exposure to contaminated blood.
6. Also children born to HIV infected mother present dilemma as antibody positivity seen upto 18 months may be due to maternal antibodies. The diagnosis in each situation can be made by direct detection method like Detection of p24 antigen
7. Detection of HIV specific DNA
8. Isolation of virus by culture.

Laboratory tests for monitoring stage and progression of HIV infection

They are classified into

Viral markers	**Immunologic markers**
Plasma HIV RNA load	CD4 count
P24 antigenemia	

Viral specific markers like antibodies to HIV antigens and soluble markers of immune activation like neopterin, beta2-microglobulin previously

recommended for monitoring the course of HIV infection are no longer recommended.

The close relationship between clinical manifestations of HIV infection and CD4+ T cell count has made measurements of the CD4 count as a routine part of the evaluation of HIV infected individuals.

CD4 T cell counts

The progressive depletion of CD4+ T lymphocytes is the cardinal event in the pathogenesis of infection by the human immunodeficiency virus. The number of these cells in the peripheral blood is the single most important parameter for monitoring the disease associated with HIV infection.

Determinants of CD4+ T-cell counts and measurements of the HIV-RNA levels in serum or plasma provides powerful tools for determining prognosis and monitoring response to therapy.

Throughout the course of the disease, the total T cell levels remain fairly constant despite a fall in CD4 T lymphocyte count, due to a concomitant rise in CD8 T lymphocytes. Therefore, the ratio of CD4 T lymphocytes to CD8 T lymphocytes is an additional important measure of disease progression. Measurement of lymphocyte subsets is done by flow cytometry which is the gold standard for enumeration of CD4 T lymphocytes.

CD4 T lymphocytes enumeration is also utilized as a surrogate marker for HIV-induced damage. The declining slope of CD4 T lymphocytes counts, indicates the speed of progression towards AIDS. While on therapy, improvement in CD4 T lymphocytes counts is indicative of the success of therapy. In resource-poor countries, with the arrival of generic drug for anti-retroviral therapy, the need of for CD4 T lymphocytes counts are the criteria for initiating ART as well as monitoring the therapeutic response in a patient.

Flow Cytometry:

Flow Cytometry uses the principle of light scattering, light excitation and emission of fluorochrome molecules to generate specific multi-parameter data from particles. The cellular suspension is incubated previously with monoclonal antibodies conjugated with different fluorochromes and it is introduced in the hydraulic system so that the cells pass individually through the flow chamber, where the laser beam intercepts the cells. This contact produces two different dispersion patterns: the frontal dispersion (Forward Scatter, A) gives us information about the cellular size, while lateral dispersion (Side Scatter, B) informs us about cellular complexity. The monoclonal antibodies linked to cellular surface is at the same time excited by the laser and generate fluorescence that are collected by photomultipliers (upto 4 at the moment) that transform the optic to

electric signal, then cytometer send all information to the computer system for later analysis.(FACS facility).

Fluorescence-activated cell sorting is a specialized type of flow cytometry. It provides a method for sorting a heterogenous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell. It provides quantitative recording of fluorescent signals from individual cells as well as physical separation of cells of interest.

CD4 cell count is extremely important in the staging of HIV infection and revised classification of the Center of Disease Control divides HIV positive patients into three categories.

CDC CLASSIFICATION SYSTEM OF HIV INFECTION

CD4+ T-cell count (cells/µL.) (CD4%)	**Asymptomatic, acute (primary) HIV or PGL**	**Symptomatic, not A or C conditions**	**AIDS-indicator conditions**
>500 (28%)	A1	B1	C1
200-499(15-28%)	A2	B2	C2
<200(14%)	A3	B3	C3

Category A: Asymptomatic HIV infection, persistent generalized lymphadenopathy (PGL).

Category B: Oropharyngeal and Vulvovaginal candidiasis, constitutional symptoms such as fever (38.5 C) or diarrhea lasting >1 month, herpes zoster (shingles).

Category C: Mycobacterium tuberculosis (pulmonary and disseminated), Pneumocystis carinii pneumonia, candidiasis of bronchi; trachea or lungs, extrapulmonary cryptococcosis, CMV, HIV-related encephalopathy, Kaposi's sarcoma, wasting syndrome due to HIV.

When the number of CD4+ T cells declines below a certain level, the patient is at high risk of developing a variety of opportunistic diseases, particularly the infections and neoplasms that are AIDS defining illness.

Opportunistic infections in HIV patients

Bacterial Infections

- Bacterial Diarrhea
- Bacterial Pneumonia
- Mycobacterium Avium Complex (MAC)
- Mycobacterium Kansasii
- Syphilis and Neurosyphilis
- Tuberculosis

Fungal Infections

- Candidiasis
- Aspergillosis
- Coccidioidomycosis
- Cryptococcal Meningitis
- Hisoplasmosis

Protozoal Infections

- Cryptosporidiosis

Malignancies

- Anal Dysplasia/Cancer
- Cervical Dysplasia/Cancer
- Kaposi's Sarcoma, Lymphomas

Viral Infections

- Cytomegalovirus (CMV)
- Hepatitis C
- Herpes Simplex Virus (oral & genital herpes)
- Herpes Zoster Virus(shingles)
- Human Papilloma Virus (HPV, genital warts, anal / cervical dysplasia/ cancer)
- Molluscum Contagiosum
- Oral Hairy Leukoplakia (OHL)
- Progressive Multifocal Leukoencephalopathy

- Isospoirasis
- Microsporidiosis
- Toxoplasmosis

Neurological Conditions

- AIDS Dementia Complex
- Peripheral Neuropathy

Others

- Aphthous Ulcers
- Thrombocytopenia
- Wasting Syndrome

BLOOD STREAM INFECTIONS

The invasion of the bloodstream by microorganisms constitutes one of the most serious situations in infectious disease and can have immediate consequences including shock, multiple organ failure, disseminated intravascular coagulation (DIC) and death. Pathogens of all the four major groups of microbes- bacteria, fungi, viruses, parasites- may be found circulating in the blood during the course of many diseases[8].

TYPES OF BLOOD STREAM INFECTIONS

The two major categories of bloodstream infections are intravascular (those that originate within the cardiovascular system) and extravascular (those that result from bacteria entering the blood circulation through the lymphatic system from another site of infection). Of note, other organisms such as fungi, may also cause intravascular or extravascular infections. However, because bacteria account for the majority of significant vascular infections, these types of blood stream infections are addressed[8].

INTRAVASCULAR INFECTIONS

Intravascular infections include infective endocarditis, mycotic aneurysm, suppurative thrombophlebitis, and intraveneous (IV), catheter associated bacteremia. These infections in the cardiovascular system are extremely serious and are considered life- threatening.

EXTRAVASCULAR INFECTIONS

Except for intravascular infections, bacteria usually enter the circulation through the lymphatic system. Most cases of clinically significant bacteremia are a result of extravascular infection.

The most common portals of entry for bacteria are the genitourinary tract (25%), respiratory tract (20%), abscesses (10%), surgical wound infections (5%), biliary tract (5%), miscellaneous sites (10%) and uncertain sites (25%). For the most part, the probability of bacteremia occurring from extravascular site is dependant on the site of infection, its severity, and the organisms including members of the family *Enterobacteriaceae*, *Streptococcus pneumonia, Staphylococcus aureus, Neisseria gonorrhoeae*, anaerobic cocci, *Bacteroides*, *Clostridium*, beta-hemolytic streptococci, and *Pseudomonas*[8].

A bacteremia or BSI is called primary if the point of entry or focus cannot be determined, or if it originates from an intravascular catheter (catheter related BSI).

Renaud and Brun- Buisson in 2001 in their recent studies, have recommended differentiating catheter -related bacteremia from both primary and secondary bacteremia. If a distant site other than an IV catheter can be established as the point of origin, BSI is referred to as secondary[27].

Another way to classify BSI is by the time or setting of acquisition, i.e. community acquired or nosocomial BSI.

A recent study by Siegman-Igra et al proposed to classify BSIs as follows : true community - acquired BSI (detected within 48h after admission in patients who have not been hospitalized within past 30 days and did not have a recent history of invasive procedures), BSI in recently discharged patients (2-30 days prior to the recent episode of BSI), BSI in patients with recent history of invasive procedures (including insertion of Foley catheters, placement of intravascular catheters, long term devices, central venous catheters, or dialysis), nursing home acquired bacteremia and true nosocomial BSI (detected > 48h after admission)[27].

PREDISPOSING FACTORS

Factors that contribute to the initiation of blood stream infections are immunosuppression, widespread use of broad spectrum antibiotics that suppress the normal flora and allow the emergence of resistant strains of microbes, invasive procedures that allow bacteria access to interior of the host, more extensive surgical procedures, and prolonged survival of debilitated and seriously ill patients[8].

PATHOGENESIS AND CLINICAL PRESENTATION

Clinical presentation ranges from benign transient bacteremia with little or no symptoms to fulminant septic shock with high mortality.

Transient bacteremia may follow manipulation of or surgery in infected or colonized areas. Intermittent bacteremia is usually seen secondary to abscesses, mostly abdominal or pelvic. Continuous bacteremia is associated with endocarditis or other intravascular infections, but may also occur in the first weeks of typhoid fever or brucellosis[59]. In patients with intermittent and continuous bacteremia, fever is the most common symptom followed by other unspecific signs of systemic disease, such as elevated heart and respiratory rate, fatigue, malaise, or failure to thrive in neonates. A substantial proportion of patients with infection may also be euthermic or hypothermic[27].

BLOOD STREAM INFECTIONS AND HIV

Bloodstream Infections (BSIs) have been a significant cause of morbidity and mortality in patients infected with the human immunodeficiency virus (HIV) [21, 22, 33, 40, 49, 58, 60, 70]. With the introduction of new antiretroviral agents and therapeutic strategies in recent years, the management of HIV-infected patients has changed profoundly. The availability of highly active antiretroviral therapy (HAART) that is capable of effectively suppressing viral replication has improved the prognosis for many HIV-infected patients over prolonged periods of time. Moreover, the use of combination antiretroviral therapy has been associated with a decline in the incidence of opportunistic infections[11,31].

Several conditions have been considered to predispose to the development of bloodstream infections in HIV-infected patients. In particular, defective cell-mediated immunity, altered B-cell function with consequent lack of serum opsonins against certain capsulated bacteria, and qualitative and quantitative deficits of neutrophils are probably the most common conditions associated with the increased incidence of bloodstream infections in HIV-infected patients.

Traditional risk factors for nosocomial bloodstream infections, such as use of invasive devices, exposure to antibiotics and duration of hospital stays, also apply to patients with HIV infection. However, the relative contribution of immunosuppression, treatment, and prevention of HIV-related opportunistic infections as risk factors for nosocomial bloodstream infection still remain to be defined[43,75]. There were no other important differences, including no difference in mortality when compared preHAART and HAART era[40].

PATHOGENS

Almost any pathogen isolated from the bloodstream as a causative agent of clinically significant bacteremia, fungemia or BSI, especially in a immunocompromised patients. The spectrum of pathogens has changed considerably over the last decades.

In recent studies of Edmond et al. 1999; Diekema et al. 2003; Wisplinghoff et al. 2004 showed, about 13% of all episodes were polymicrobial, 60-65% were caused by gram positive and 25-31 percent by gram negative organisms. Fungi, mainly *Candida* species, were isolated from 7-10% of episodes. Anaerobic bacteria accounted for about 1percent of BSI). The most common pathogens isolated from BSI are Coagulase negative staphylococci, *Staphylococcus aureus, Enterococci, Candida spp., E.coli, Klebsiella spp., P.aeruginosa, Enterobacter spp., Serratia spp., A.baumanni*[27].

GRAM POSITIVE ORGANISMS

Staphylococcus aureus

S.aureus is a major cause of morbidity and mortality. Commonly isolated from patients with both community-acquired and nosocomial BSI usually originates from a localized infection, an intravascular catheter or a contaminated syringe (e.g. in IV drug users). Methicillin resistance, first detected in the 1960s (Barber 1961), emerged as a major clinical and epidemiological problem in hospitals in the 1980s.

Studies by Frank et al., Fergie and Purcell, Groom et al., Naimi et al., and Baggett et al. showed that, even though MRSA has been a nosocomial pathogen, there are an increasing number of reports of MRSA BSI in

patients with little or no association to the healthcare systems, suggesting the emergence of true community-acquired MRSA.

Small colony variants (SCV), a naturally occurring subpopulation of *S.aureus* have also been identified as a cause of BSI (Seifert et al. 2003b). *S.aureus* SCVs have been implicated in persistent and recurrent infections that give a poor clinical and bacteriological response to standard antimicrobial therapy in patients with BSI, chronic osteomyelitis, cystic fibrosis, and AIDS particularly after prolonged exposure to antibiotics (von Eiff et al. 1997; Kahl et al. 1998; Proctor et al. 1998)[27].

Coagulase-negative staphylococci

BSI caused by Coagulase –negative staphylococci is mostly nosocomial, but about 8% have also been community acquired (Diekema et al. 2003). CoNS were being reported from cases only if isolated from two or more separate blood cultures. *S.epidermidis* is the most commonly isolated species among CoNS, others implicated in BSI includes *S.haemolyticus, S.capitis, S.lugdunensis, S.saprophyticus, S.schleiferi, S.simulans* and *S.warneri*[27].

Enterococcus species

Primary BSIs are generally seen in highly compromised or immunosuppressed patients and may be due to inapparent bacterial

translocation from a gastrointestinal source (Shlaes et al. 1981b; Graninger and Ragette 1992; Linden et al.1996) or intravascular catheters[27].

Other gram –positive organisms

Streptococcus pneumonia, S. viridans, Listeria monocytogenes, and Diphtheroids.

GRAM-NEGATIVE ORGANISMS

***Escherichia coli* and other Enterobacteriaceae**

Enterobacteriaceae are important causes of both community acquired and nosocomial infections including BSI, accounting for 17 percent of all BSI, making them the second most common cause of BSI after CoNS. *E.coli* accounts for 7-11% of nosocomial BSI to date (Pittet and Wenzel 1995; Edmond et al. 1999; Wisplinghoff et al.2004), *Klebsiella spp.* causes about 10 percent of community acquired BSI and about 6 percent of nosocomial BSI. Non-typhoid *Salmonella* species have been described as etiological agents of community acquired BSI in both immunocompromised and previously healthy patients[27].

Pseudomonas aeruginosa

P.aeruginosa BSI is mainly nosocomial, contributing significantly to morbidity and mortality of hospitalized patients. Nosocomial pneumonia due to *P.aeruginosa* is often associated with BSI in adult and pediatric AIDS patients; the prognosis is poor with fulminant progression to septic shock

and death occurring within 3-4 days after the onset of pulmonary symptoms[27].

Other gram-negative pathogens

Acinetobacter baumannii, Haemophilus influenza, Neisseria species

FUNGI

Blood cultures are an important tool in the diagnosis of disseminated fungal infections. Lysis centrifugation system appears to be more sensitive method for detection of filamentous fungi as well as *Histoplasma capsulatum* and other dimorphic fungi. *Candida* species, particularly *C.albicans* are frequently isolated from the blood stream, accounting for up to 10 percent of all nosocomial blood culture isolates and is usually associated with underlying conditions such as malignancies, neutropenia, HIV/AIDS[27].

LABORATORY DIAGNOSIS OF BLOOD STREAM INFECTIONS

Blood cultures are valuable diagnostic tool and should be considered in an broader range of situations in compromised patients with underlying conditions that predispose for BSI[27]. Bacterial growth can be detected using techniques ranging from manual to totally automated methods. Once growth is isolated, identified, and tested for its susceptibility to various antimicrobial agents when appropriate[8].

SPECIMEN COLLECTION AND TRANSPORT

The blood culture denotes a specific volume of blood which is aseptically drawn by a single venipuncture, inoculated into culture medium or media and investigated for the presence of microorganisms. Obtaining blood cultures from indwelling intravascular catheters is generally not recommended, unless the involvement of the catheter as a source of BSI is to be investigated[27].

SPECIMEN COLLECTION

Since the rapid and reliable recovery of even small numbers of bacteria from the blood is the primary goal of blood cultures, the media also provide excellent growth conditions for contaminants and contamination poses a serious problem. In addition, an increasing number of true bacteremia and BSI in immunocompromised patients is caused by bacteria that are part of the common skin flora, such as coagulase negative staphylococci, corynebacteria, and *Bacillus* species. Correct interpretation of such findings largely depends on the assumption that proper aseptic techniques have been used for specimen collection and transport.

COLLECTION SITE

Usually blood for culture is drawn from a peripheral vein, e.g. vena cubitalis. Contamination is more likely if femoral vein or a vein in close proximity to an inflammation or skin infection site is used (Washington

1975). Arterial blood does not offer any advantage with regard to detection of pathogens, not even for endocarditis or fungemia (Tenney et al. 1982; Vaisanen et al. 1985). The reliability and diagnostic impact of blood cultures drawn through an intravascular catheter is controversial (Tonnesen et al.1976; Bryant and Strand 1987). Contamination rates are higher and catheters may be colonized with organisms that do not necessarily invade the blood stream. However blood from the source, even via different lumens of the catheter, may be used in conjunction with peripheral blood samples to assess the presence of a catheter related BSI[27].

ANTISEPSIS

Once a vein is selected, the skin site is disinfected with 70% isopropyl alcohol in a circle approximately 5cm in diameter, rubbing vigorously. Starting from the centre of the circle, 2% tincture of iodine (or povidone-iodine) in ever widening circles until the site is saturated with iodine and allowed to dry on the skin for at least 1 minute. Gloves should be worn by the phlebotomist[42].

TIME OF COLLECTION

To obtain the maximum yield of organisms, blood should be collected immediately before the onset of chills. It is generally recommended to draw blood for culture when the patient's temperature rises, as soon as possible after the onset of fever and chills. Whenever possible, blood cultures should

be obtained before antimicrobial chemotherapy is initiated. If a patient is already receiving antimicrobials, blood cultures should be drawn towards the end of a dosing interval[42].

SPECIMEN VOLUME

Adults. Most bacteremias in adults have a low number of colony forming units (CFU) per milliliter of blood. Therefore a sufficient sample volume is critical for the successful detection of bacteremia[35]. The greater the chance of isolating the organism, when more blood is cultured; findings from a study suggested that the yield increases by 3.2% for each milliliter of blood cultured[42]. Therefore, collection of 10-20mL of blood per culture is strongly recommended for adults. Volumes of 20 mL increased the yield by 30% compared with 10mL volumes[18].

Children. It is not safe to obtain large volumes of blood from children, particularly infants; in light of low level of bacteremia in infants and children and based on the premise that it is safe to obtain as much as 4% to 4.5% of a patients known blood volume for culture and on the known relationship between blood volumes for culture from infants and children (Table 1) have been made by Baron and colleagues[6].

Table 1 Blood Volumes Suggested for Cultures from Infants and Children

Weight of patient	**Recommended volume of blood for culture (mL)**				
Kg	**Total blood volume**	**Culture No 1**	**Culture No 2**	**Total volume for culture**	**% of total blood volume**
≤1	50-99	2		2	4
1.1-2	100-200	2	2	4	4
2.1-12.7	>200	4	2	6	3
12.8-36.3	>800	10	10	20	2.5
>36.3	>2200	20-30	20-30	40-60	1.8-2.7

NUMBER OF BLOOD CULTURES

The detection rate increases with the number of blood cultures obtained. In patients with BSI found 99 percent sensitivity if only two cultures were drawn[4, 30, 46, 67, 69]. There is currently no recommendation for an optimal time difference between the first and the second culture[12].

CULTURE MEDIA

Depending on the indication, the patient, and the suspected pathogen, the use of special culture media or a different number of bottles per blood culture may be useful. Nutrient rich broth in a variety of formulations can be used to culture microorganisms from the blood. These include brain-heart

infusion broth (BHI), tryptic –soy-broth (TSB), soybean casein digest broth, peptone broth and Columbia media, which may be supplemented with haemin or NADH[8]. Microbial growth in human blood is inhibited by a number of substances including antibodies, leukocytes, complement, lysozyme, and antimicrobial substances, which about one third of patients already receive, when blood cultures are obtained (Weinstein et al. 1997). Blood clots also reduce the diagnostic yield, thus blood drawn for culture may be either inoculated into the blood culture broth media or sterile blood culture bottle containing an anticoagulant.

Sodium polyanethol sulfonate (SPS, Liquoid) (Wilson et al. 1994), in concentrations of 0.025% to 0.03% is the best anticoagulant for blood, also inhibits lysozomal activity, complement and phagocytosis (Traub and Kleber 1977; Chandrasekar and Brown 1994)[66]. Also 1:5 to 1:10 dilutions of blood enhances detection by neutralizing the bactericidal activity of human serum[8] and by diluting antimicrobial agents to sub inhibitory concentrations.

Aminoglycosides and polymyxins are also inhibited by SPS. Side effects of SPS include some inhibition of the growth of *Neisseria gonorrhea, Neisseria meningitides, Gardenella vaginalis, Peptostreptococcus anaerobius* and *Moraxella catarrhalis*. Antimicrobial agents can also be neutralized b y adding unspecific adsorbants such as antimicrobial disease(ARD) (Peterson et al. 1983), antibiotic binding resins on glass beads (BACTEC) (BD,

Sparks, MD), or activated charcoal and Fuller's earth (Ecosorb, BacT/ALERT, BioMerieux Inc., Durham, NC).

TRANSPORT

Rapid transport of blood cultures to the microbiology laboratory is mandatory. Refrigeration of blood cultures should be avoided. In the laboratory, all blood cultures should be processed immediately[68].

SAMPLE PROCESSING

In patients with BSI, the appropriateness of antimicrobial chemotherapy is correlated with survival (Ibrahim et al.2000). Processing of blood cultures generally includes incubation, gram staining and subcultures. Manual processing and automated blood culture systems are used.

CONVENTIONAL BLOOD CULTURES

All manual processing should be done under a protective hood, both to avoid contamination and to protect laboratory personnel. All blood cultures should be incubated for at least 6-18h at 36±1°C before processing. Afterwards blind subcultures and microscopic examination of all aerobic cultures should be performed, using approximately 0.2 mL of broth, which should be removed under sterile aseptic precautions after gentle shaking.

For microscopic examination, Gram or acridine-orange stains can be performed. While acridine orange stains detected 10^4CFU/mL, Gram stain detected only 10^5 CFU/mL (Seifert et al.1997).

Blood agar, chocolate agar and some differential media (e.g. MacConkey) should be used for subculture of blood and subsequently incubated at 36±1°C in an aerobic atmosphere with 5- 10 % CO_2. Also if signs of turbidity, change of color, or gas production are detected, the respective culture bottle must be processed[8].

Blood cultures do not need to be routinely incubated for more than 7 days for suspected bacterial cultures[42]. Fungal blood cultures were held for 21 days before being reported as negative[59].

LYSIS CENTRIFUGATION SYSTEM

Lysis centrifugation system commercially available is the Isolator (Wampole Laboratories, Cranbury, NJ). The Isolator consists of a stoppered tube containing saponin to lyse blood cells, polypropylene glycol to decrease foaming, SPS as an anticoagulant, EDTA to chelate calcium ions and thus inhibit complement cascade and coagulation and a small amount of an inert fluorochemical (Fluorinet, 3M Co., St. Paul, Minn) to cushion and concentrate the microorganisms during 30minute centrifugation, the supernatant is discarded, the sediment containing the pathogen is vigorously vortexed, and the entire sediment is plated to solid agar[8].

AUTOMATED CULTURE SYSTEMS

The first automated blood culture system, BACTEC 460 (Becton Dickinson), was introduced in the 1970s. The use of automated blood culture

systems rapidly increased after the introduction of continuous monitoring blood culture systems in the early 1990s. In all systems cultures are monitored at intervals between 10 and 24 min, 24 h per day, shortening time to detection by 1-1.5 days[53].

BACTEC Systems

The BACTEC system measures the production of carbon dioxide (CO_2) by metabolizing organisms. While the first BACTEC systems used ^{14}C labeled substrates, the newer BACTEC 9000 series use fluorescence sensing mechanism [63].

BacT/ALERT Microbial detection system:

BacT/ ALERT (BioMerieux, Durham, NC), measures CO_2 derived pH changes by a colorimetric sensor in the bottom of each bottle. The sensor is separated from the broth medium by a membrane that is permeable only to CO_2. As the organisms grow, they release CO_2, which diffuses across the membrane and is dissolved in water present in the matrix of the sensor. As CO_2 is dissolved hydrogen ions are generated which cause color change in the sensor (blue to light green to yellow as the pH decreases); this color change is read by the instrument.

ESP system

In ESP culture system II (Trek Diagnostic systems, Inc., Cleveland, Ohio),microbial growth is detected by monitoring changes in head space

pressure by a sensitive detector that is attached to the blood culture bottles, by consumption and/or production of gases as organisms metabolize nutrients in the culture medium.

HANDLING POSITIVE BLOOD CULTURES

All clinically significant isolates should be identified to species level with biochemical reactions or by rapid identification systems.

ANTIMICROBIAL SUSCEPTIBILITY TESTING:

In a recent study by Ibrahim et al., mortality was double when inappropriate empirical antibiotics were administered to patients with BSI. Therefore successful management must include early and appropriate antimicrobial therapy in addition to supportive care and elimination of a removable focus, if present.

ANTIBACTERIAL SUSCEPTIBILITY TESTING:

Antibiotic sensitivity testing was performed by the Kirby-Bauer disc diffusion technique, using 0.5 McFarland's turbidity as the standard inoculum's density on Mueller Hinton agar plates. Commercial Hi-Media antibiotic discs were used.

ANTIFUNGAL SUSCEPTIBILITY TESTING:

The recent increased incidence of fungal infections and the growing number of new antifungal agents have multiplied the demand and interest for invitro antifungal susceptibility testing.

MANAGEMENT OF BSI

Le and Bayer et al recently reviewed the general treatment principles to be followed: 1) high dose parenteral antimicrobial therapy is recommended to reach sustained antibacterial activity; 2) prolonged administration of antimicrobial therapy is necessary to prevent relapse; 3) bactericidal antimicrobial agents are generally preferred over bacteriostatic agents; 4) antibiotic combination therapy is recommended to produce a rapid bactericidal effect. The choice of antibiotics for the treatment is governed by the susceptibility of the causative organism. Determination of minimal inhibitory concentration is recommended to define optimal treatment.

MATERIALS AND METHODS

STUDY PERIOD

This is a cross sectional study undertaken over period of June 2009 – May 2010

STUDY PLACE

This study was carried out at the Institute of Microbiology, Madras Medical College, Chennai and Antiretroviral therapy (ART) centre, Madras Medical College, and Government General Hospital, Chennai.

ETHICAL CONSIDERATIONS

This study was reviewed and approved by Institutional Ethical committee, Madras Medical College & General Hospital, Chennai 3. Informed written consent to participate in the study was obtained from the patients or their guardians after providing full explanation of the study. All data were handled confidentially and anonymously.

STATISTICAL ANALYSIS

Statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) and Epi-Info softwares by a statistician. The proportional data of this cross sectional study were tested using Pearson's Chi Square analysis test and Binomial proportion test.

STUDY GROUP

Cases:

Study group includes 100 HIV positive adult inpatients irrespective of the symptomatology, CD4 count and ART status.

Inclusion Criteria

1. HIV positive patients: Seropositivity status of the patients was determined as per NACO guidelines.
2. Patients above 15 years of age group (For the purposes of HIV case definitions for reporting and surveillance, children are defined as younger than 15 years of age and adults as 15 years or older)[71].
3. Both Males and Female were included in the study.

All patients satisfying the inclusion c riteria were only once documented and were assigned serial numbers. Patients were interviewed by structured questionnaire and their hospital records were used to know about their past medical conditions. They were prospectively followed up until discharged or 30 days after admission.

Controls:

Control group includes 50 patients, seronegative for HIV admitted as inpatients, irrespective of the symptomatology.

Case Definition

Bacteremia or blood stream infection (BSI) was diagnosed when a blood culture grew an organism with (secondary BSI) or without (primary BSI) any obvious focus of sepsis. True bacteremia was considered when one or more blood cultures showed a recognized pathogen.) .

Community-acquired bacteremia was defined if the first positive blood culture was obtained before or within 48 hours of hospitalization.

Nosocomial bloodstream infections were considered, if signs and symptoms of these infections became evident >48 hours following hospital admission and/or if the patient had been hospitalized during the 2 weeks before the current admission.

The source of bacteremia was identified by the isolation of the same pathogenic organism from both the source and the blood.

The following definitions were used to categorize the source of the patients' bacteremia.

Pneumonia was defined as the presence of an acute illness with respiratory symptoms and an infiltrate on chest roentgenography.

Cellulitis required the physical finding of an erythema, tenderness, and warmth in a focal distribution.

Phlebitis was defined as an inflammation around a venous line site, a positive line culture or a catheter in place at least 72 hours and the absence of another source of bacteremia.

Endocarditis was defined as the demonstration of valvular vegetations on echocardiography, evidence of septic emboli or a new murmur, and the absence of another source of bacteremia.

A **urinary tract infection** required a positive urine culture result and no other source of the bacteremia.

Catheter- related blood stream infection (CRBSI) was diagnosed when blood culture from a peripheral vein and CVC grew an organism and the CVC tip with quantitative bacterial counts >15 CFU (Maki method).

DATA COLLECTION

All patients underwent history and physical examination. Information on antimicrobial therapy before admission was recorded. The stage of HIV infection was recorded using the WHO Clinical Staging System[74].

SPECIMEN COLLECTION

Blood cultures were obtained using aseptic technique. Venipuncture sites were disinfected before phlebotomy with 0.5% chlorhexidine solution followed by 70% isopropyl alcohol[72].

For the purpose of the culture, 10 ml of blood was drawn, and was used to inoculate biphasic brain-heart infusion (BHI) bottles and trypticase soy medium.

For estimation of CD4 counts, 3 ml of blood collected in EDTA containing vacutainers, Counting done using BD FACS Counter. Peripheral smears were also made from blood samples for evaluation of parasites: Plasmodium and Microfilaria.

Blood culture bottles were incubated at 37° C for 24 hours. Subcultures were done at the appearance of turbidity, gas production or the presence of microcolonies over the clot at 24 hours, blind subcultures at 48 hrs irrespective of turbidity or gas production and final subcultures were done at the end of 7th day, as follows: a) on 5% sheep Blood Agar aerobically.

b) On 5% Blood Agar Anaerobically,

c) On MacConkey Agar Aerobically,

d) Nutrient Agar

e) On Chocolate Agar in CO2, all incubated at 37°C for 24 hours &

f) 2 Sabouraud Dextrose Agar tubes incubated at 25°C and 37°C.

CULTURE IDENTIFICATION

Colony morphology on blood agar, MacConkey agar, chocolate agar at 18-24 hours were noted and isolates were identified by

1. Gram stain- to identify Gram positive and Gram negative organisms.
2. Gram-positive cocci were proceeded with catalase and coagulase tests.
3. Hanging drop- to find out motile and non motile organisms.
4. Preliminary tests like Oxidase, Catalase were performed.
5. Members of the species were identified based on biochemical parameters using IMViC reaction and sugar fermentation medium.

ANTIMICROBIAL SUSCEPTIBILITY TESTS:

Antimicrobial susceptibility of the isolates were tested using Kirby Bauer disc diffusion technique as per CLSI guidelines[16].

Medium used: MHA (Muller Hinton Blood agar plate)

Inoculum: 0.5McFarland turbidity, lawn Culture.

Incubation: 37˚C ambient air, incubated for 16-18 hrs

Preparation of inoculums:

About 4-5 colonies of the same morphology were picked up with straight wire and inoculated in 5ml of peptone water, incubated at 37°C for 3-5 hrs to attain 0.5 McFarland's turbidity[8]. A sterile cotton swab was dipped into it and pressed firmly against the wall of the test tube to remove excess broth from the swab.

Dried surface of Mueller Hinton Agar plate was swabbed in three directions approximately 60° each time to ensure an even and complete distribution of the inoculum over the entire plate. The anti microbial discs

were dispensed on the agar plate and pressed down to ensure complete contact with agar surface. Discs were distributed evenly so that they were not closer than 24mm from centre to centre. Not more than 6 discs were placed in the plate[32]. After 16-18 hrs of incubation each plate was examined. The diameter of the zones of complete inhibition was measured including the diameter of the discs. Zones were measured to the nearest whole 'mm' using a ruler which was held on the back of the inverted Petri plate.

Control strains used were

1. *Staphylococcus aureus ATCC 25923*

2. *Escherichia coli ATCC25922*

3. *Pseudomonas aeruginosa ATCC 29823*

Growth in Sabourauds Dextrose Agar is subjected for following tests:

The Sabourauds dextrose agar slopes were examined after 48hrs, 96 hrs, 5days, 7days, 14days and one month for the appearance of yeasty or mouldy growth[34].

GRAM STAIN

Gram positive oval budding yeasts cells with presence or absence of pseudohyphae

INDIA INK PREPARATION

A loopful of creamy colonies from SDA was taken and mixed with a drop of India Ink over a slide. Coverslip is applied over it and focused under high power objective to see budding yeast cells with capsule.

GERM TUBE TEST

A loopful of creamy white yeast like colony from SDA was taken and it was inoculated in to 0.5ml of mammalian serum. It was incubated at 37 degree centigrade for one and a half to 2 hours. After incubation period, a loop full of this serum suspension was placed on a clean glass slide and covered with cover slip and focused under high power objective to see the characteristic germ tube formation.

CHROM AGAR (HIMEDIA)

A single colony from Sabourauds dextrose agar was taken and it was streaked on chrom agar and incubated at 37°C for 48 hrs. After incubation period the plates were observed for characteristic coloured colonies of candida.

CORNMEAL AGAR

A single colony from Sabouraud's dextrose agar was inoculated on to plate of cornmeal agar containing 1%Tween 80 and trypan blue. Three parallel steaks were made about half an inch apart at a 45 degree angle to the culture medium. A sterile coverslip was placed over it and incubated at 30

degree centigrade for 48 hrs. After incubation the areas where the cuts into the agar[36] were made were examined for the presence of blastoconidia, arthroconidia, pseudohyphae, hyphae or chlamydoconidia[8].

ANTIFUNGAL SUSCEPTIBILITY TESTS:

The antifungal susceptibility tests was done by two methods

1) DISC DIFFUSION METHOD

2) BROTH MICRODILUTION METHOD

Inoculum preparation:

The inoculum suspension was prepared by picking five colonies, each of atleast 1mm in diameter, from 24 hour old cultures of *candida* species and suspending the material in 5ml of sterile saline. The suspension was then adjusted spectrophotometrically at 530nm to match the transmittance produced by 0.5 McFarland's barium sulphate standard. This procedure produces an inoculums size 1×10^6 to 5×10^6 CFU/ml. The same inoculum was used for both methods.

The following standard strain was tested each time to ensure quality control:

Candida albicans ATCC 90028

Disc diffusion method

It was performed on Muller Hinton agar plate supplemented with 2% glucose and 0.5µg/ml methylene blue[17]. Antifungal susceptibility testing was

carried out following the M 44-A. National Committee for Clinical Laboratory Standards (NCCLS) guidelines, using fluconazole and itraconazole antifungal discs[37].

Enumeration of CD4 + T cells

Estimation of CD4+ T lymphocyte of the HIV infected patients was done by Fluorescent Activated Cell Sorter (FACS) count system.

- Blood sample was collected aseptically in a K3 EDTA Vacutainer tube.
- 50µl of whole blood was added to the reagent tube containing fluorescent labelled antibodies which results in the binding of labelled monoclonal antibodies to the lymphocyte surface antigen.
- After adding fixative solution to the reagent tube, the sample was run in the instrument[38].
- The cell comes in contact with the laser beam which causes the fluorochrome labelled antibodies to fluoresce.
- The fluorescent light provides the information necessary for the instrument to count the cells.
- The software identifies the T- lymphocyte subpopulation and correlates with the absolute count
- Results provide the absolute counts of CD4+ lymphocyte[19].

PERIPHERAL SMEAR EXAMINATION FOR MALARIAL PARASITES

Peripheral smear examination for malarial parasite is the gold-standard in confirming the diagnosis of malaria. Thick and thin smears prepared from the peripheral blood are used for the purpose.

PREPARATION OF THE SMEAR[13, 65]

Thin Smear

- One end of the slide is allowed to touch the top of the blood drop on the patient's finger.
- Only the top of the drop should come into contact with the slide.
- The quantity of blood to be transferred to the slide should not exceed 1.5 ml, usually corresponding to a diameter of 3-4 mm.
- The edge of a second slide (or a cover slip) is then laid on the drop of blood that will spread on the entire line of contact between the two slides.
- The second slide, steadily held to form a 45° angle with the original slide, is then moved to the opposite end of the slide to which the drop was originally located.
- In the well prepared thin film, the blood film should end with multiple tails not touching the edges of the slide.

- Red blood cells should be visible one by one without overlapping.Abnormally thick slide may be the result of an exaggerated volume of blood or of an angle larger than 45°.

Thick smear

- A volume of blood twice to thrice the one used for a good thin film is needed for the preparation of a thick film (3.0 to 4.0 ml). This is usually accomplished by touching three times the top of the blood drop at the top of the finger to obtain a triangle on the slide.
- The blood is then gently mixed for 20-30" using the corner of a second slide to defibrinate the blood and to obtain a round smear of about 1 cm in diameter.
- The thickness of the obtained film should allow the reading of the printed text of a usual newspaper through it (Gilles, 1993)

Staining:

Thick films are ideally stained by the Giemsa's stain for screening of parasites. Thin blood films stained by Giemsa's or Leishman's stain are useful for specification of parasites

PROCEDURE

The thick film was first de-hemoglobinised in water and then stained with Giemsa.

Rapid Giemsa:

- Prepared a 10% Giemsa in buffered water at pH 7.1.
- Immersed the slide in the stain for 5 minutes.
- Rinsed gently for 1 or 2 seconds in a jar of tap water.
- Drained, dried and examined.

Thin film was stained with Leishman's stain.

Leishman's stain:

- Added 7-8 drops of the stain and left for 1-2 minutes.
- Then added 12-15 drops of buffered distilled water, mixed thoroughly, left for 4 - 8 minutes.
- Then washed off with clean water, drained, dried and examined.

INTERPRETATION OF RESULTS

A culture was considered significant when a pure isolate was isolated from a pair of blood culture bottles. Samples that showed no growth in BHI media and media were discarded after 7days. Fungal blood cultures were held for 21 days before being reported as negative. Blood isolates of *Staphylococcus* spp. were considered to be contaminated if a single blood culture yielded the organisms, if the clinicians judged the organisms to be contaminants, and if antibiotic therapy directed against these organisms was not administered.

RESULTS

A total of 150 individuals were enrolled in this study, conducted during May 2009 to May 2010 at Institute of Microbiology, Madras Medical College, Chennai. It included 100 HIV seropositive cases and 50 HIV seronegative individuals as controls, determined by HIV antibody detection methods. Blood samples collected from all the subjects were subjected to culture in Brain Heart Infusion Biphasic medium and Trypticase Soy broth. Peripheral smear was stained by Leishmann staining and enumeration of CD4 counts were done. Results were analyzed further as follows:

TABLE 2: DESCRIPTION OF STUDY GROUPS

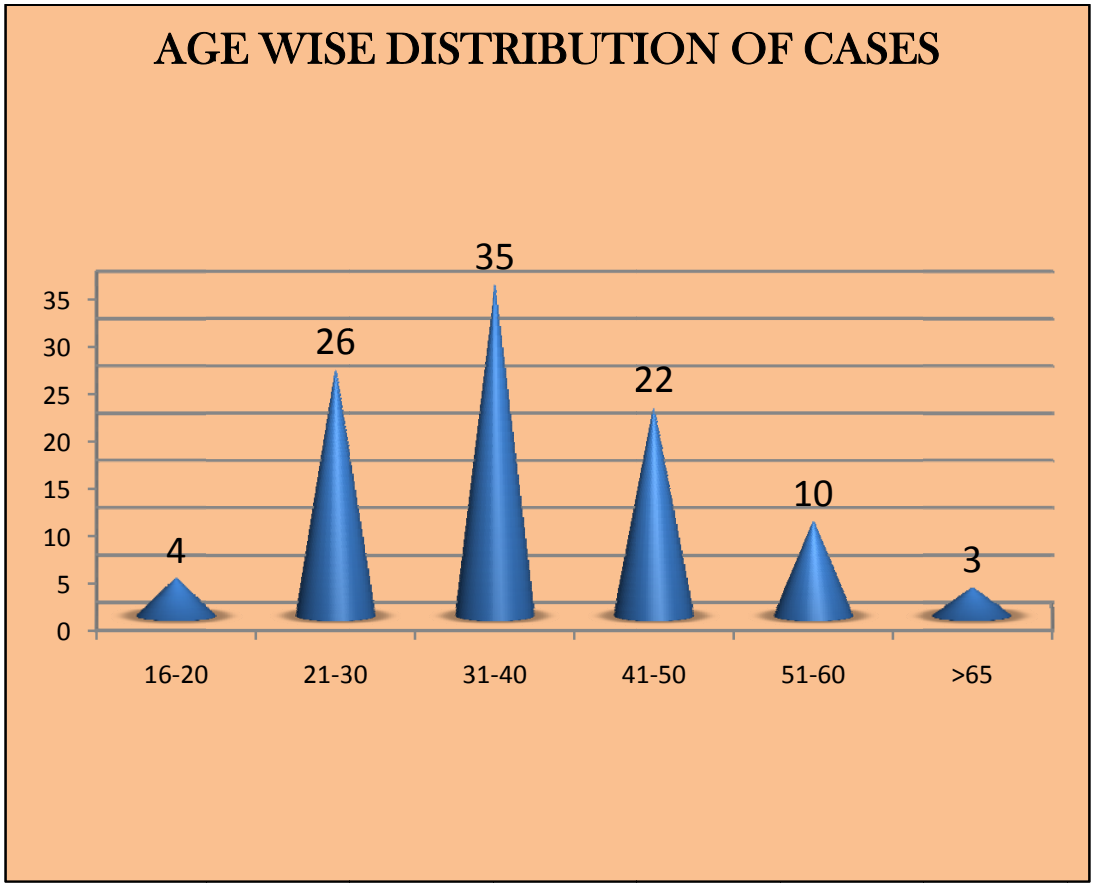
Study Group	Description	Number of individuals
Cases	HIV seropositive patients with or without fever	100
Controls	Seronegative for HIV	50

Table 4: AGE AND SEXWISE DISTRIBUTION CASES AND CONTROL

AGE	CASES(n=100)			CONTROL(n=50)		
	Male(%)	Female(%)	%	Male(%)	Female(%)	%
16-20	3(4.2)	1(3.3)	4	4(14.2)	3(13.6)	14
21-30	16(22.8)	10(33.3)	26	4(14.2)	7(31.8)	22
31-40	24(34.3)	11(36.7)	35	7(25)	6(27.3)	28
41-50	18(25.7)	4(13.3)	22	3(10.7)	3(13.6)	12
51-60	7(10)	3(10)	10	3(10.7)	1(4.5)	10
>65	2(2.8)	1(3.3)	3	7(25)	2(9.09)	14
	70	30		28	22	

Both the study groups included individuals >16 years and both sexes. Cases included 70 males and 30 females and control group had 28 males and 22 females.

Majority of the male cases in the study population belonged to the age group of 31-40 years (28%) and in control group, 31-40years (25%) and >65 years(25%) were commoner. Males outnumbered female in both cases and controls.


AGE WISE DISTRIBUTION OF CASES
35
30
25
20
15
10
5
0
4
26
35
22
10
3
16-20
21-30
31-40
41-50
51-60
>65

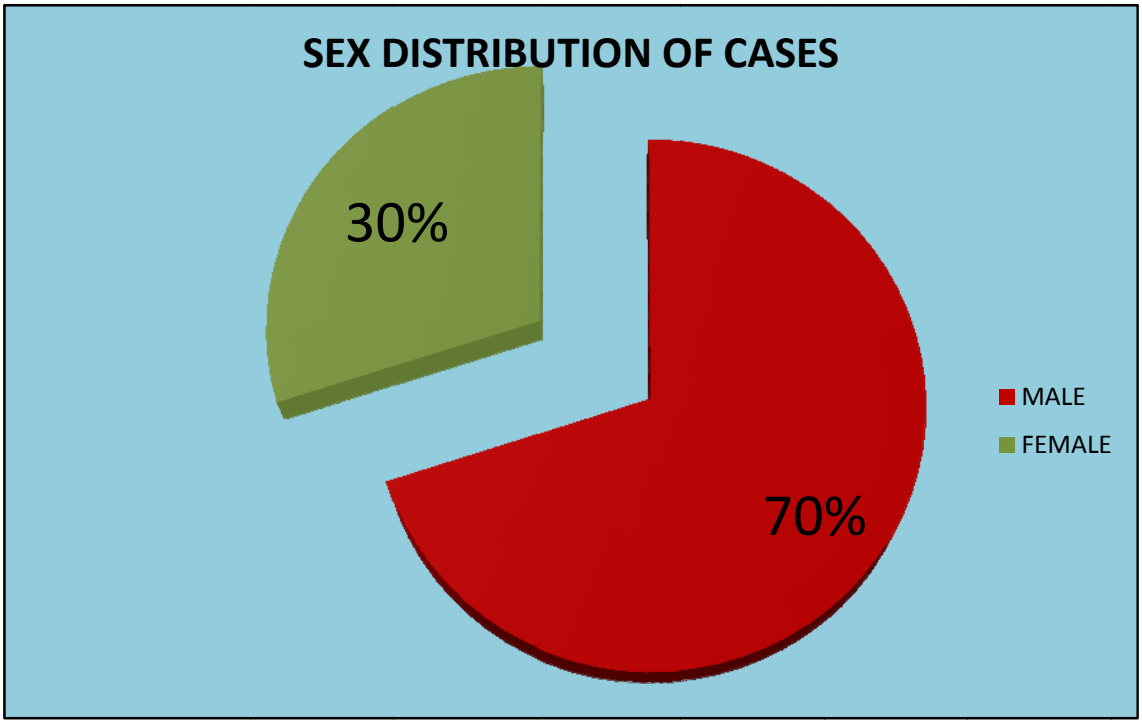

SEX DISTRIBUTION OF CASES
30%
70%
MALE
FEMALE

Table 4: DEMOGRAPHIC DISTRIBUTION OF CASES

	MALE	FEMALE	TOTAL
RURAL	42	24	66
URBAN	28	6	34
TOTAL	70	30	100

66% of cases were reported from rural population and 34% cases from urban population.

Table 5: EDUCATIONAL STATUS

OCCUPATION	MALES (n=70)	%	FEMALE (n=30)	%	Total %
Illiterates	13	18.5	11	36.6	24
Primary school	15	21.4	7	23.3	22
High school	18	25.7	5	16.6	23
Higher secondary	12	17.1	6	20	18
Graduates	7	10	1	3.3	8
Technical	5	7.1	-	-	5
Total	70		30		100

Illiterates contributed about 24% of study population

Table 6: RISK BEHAVIOUR OF CASES

S NO	RISK BEHAVIOR	MALE (n=70)	%	FEMALE (n=30)	%	TOTAL (%)
1	Smoking	52	74.2	-	-	52
2	Alcohol	47	67.1	-	-	47
3	Multiple partners	58	82.8	2	6.6	60
4	Homosexual behavior	6	8.5	-	-	6
5	Spouse positive	56	80	28	93.3	84
6	VDRL positive	32	45.7	6	20	38

74.2% and 47% of male population were indulged in smoking and alcoholism respectively. Homosexual behavior was observed in 8.5% study population.

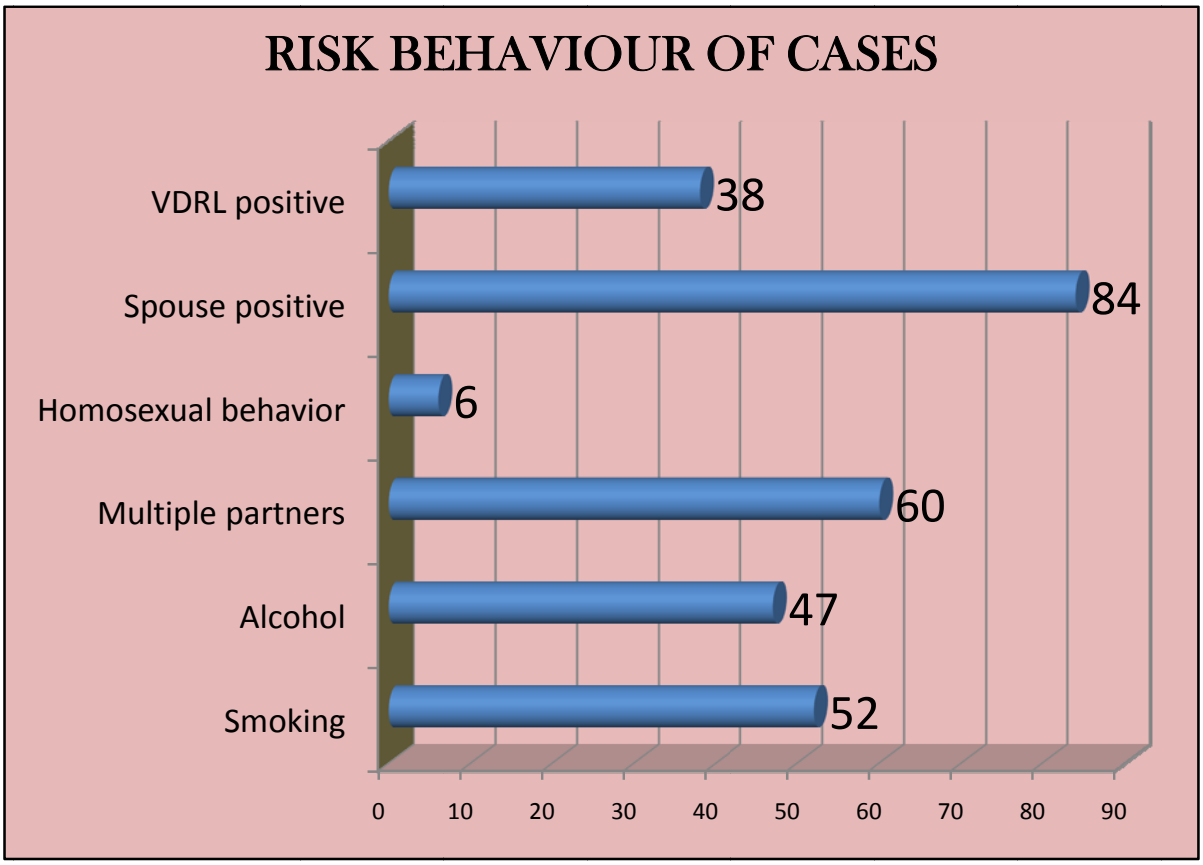

RISK BEHAVIOUR OF CASES
VDRL positive
Spouse positive
Homosexual behavior
Multiple partners
Alcohol
Smoking
38
84
6
60
47
52
0
10
20
30
40
50
60
70
80
90

Table 7: STAGING OF THE DISEASE (WHO staging, 2007):

STAGE	MALE (n=70)	%	FEMALE (n=30)	%	TOTAL (%)
I	10	14.2	7	23.3	17
II	12	17.1	1	3.3	13
III	19	27.1	13	43.3	32
IV	29	30	9	30	38

Most of the cases were in stage IV disease (38%)

Table 8: CD4 DISTRIBUTION AMONG THE CASES

CD4 COUNTS	MALES (n=70)	%	FEMALES (n=30)	%	Total (%)
<50	5	7.1	1	3.3	6
51-100	8	11.4	4	13.3	12
101-200	19	27.1	5	16.6	24
201-500	23	32.8	16	53.3	39
501-750	8	11.4	2	6.6	10
>750	7	10	2	6.6	9

39% of cases were having CD4 counts between 201- 500.

Table 9:

DISTRIBUTION OF CO-MORBID ILLNESS IN HIV INFECTED CASES

S no	Co-morbid illness	Male (n=70)	%	Female (n=30)	%	Total (%)
1	Hypertension	17	24.2	5	16.6	22
2	Diabetes Mellitus	10	14.2	2	6.6	12
3	Asthma	7	10	10	33.3	17
4	Cirrhosis	5	7.1	-	-	5
5	Renal disease	10	14.2	1	3.3	11
6	Previously treated for TB	27	38.5	8	26.6	35

Majority of cases had been previous treated for tuberculosis (35%). Diabetes mellitus and renal diseases were associated with 12% and 11% of study subjects.

Table 10: CLINICAL SYMPTOMATOLOGY CORRELATION WITH BSI

S no	Clinical features	No of Patients(n=100)	%	No of pts with BSI(%)
1	Fever	30	30	15(65.2)
2	Wt Loss >10%	75	75	23(100)
3	Generalised Lymphadenopathy	17	17	10(43.4)
4	Persistent Cough	24	24	12(52.2)
5	Persistent Diarrhoea	50	50	13(56.5)
6	Genital Ulcers	12	12	2(8.6)
7	Oral Thrush	29	29	9(39.1)
8	Skin Lesions	29	29	13(56.5)
9	Anemia	78	78	12(52.2)
10	Jaundice	34	34	6(26.1)
11	Neurological Symptoms*	18	18	4(17.3)
12	Tubercular infection	23	23	12(52.1)

* Includes headache, neck stiffness, hemiparesis, hemiplegia.

Fever was presenting symptom in only 30% of cases. 23% of cases were on treatment for tuberculosis.

Table 11: PREVALENCE OF ETIOLOGIC AGENTS

Study group	Total	Positive	%
Cases	100	23	23
Controls	50	7	14
Total	150	30	20

P= 0.0062

23 isolates were obtained from cases and 7 isolates from control group. In statistical analysis, by Pearson Chi-square test, P =0.0062 .This is statistically significant indicating the increased prevalence of infection in HIV patients.

Table12: EPIDEMIOLOGY OF BSI IN CASES AND CONTROLS

	HIV infected Cases (n=23)		**HIV uninfected controls(n=7)**	
	No of BSI episodes	**%**	**No of BSI episodes**	**%**
Community acquired	18	78.3	4	
Nosocomial acquired	5	21.7	3	

P=0.538

21.7% of cases were nosocomial acquired and 42.9% of controls were found in nosocomial settings. In statistical analysis, by Pearson Chi- Square test, P=0.538, which is statistically insignificant, showing no difference in epidemiology of infections between cases and controls.

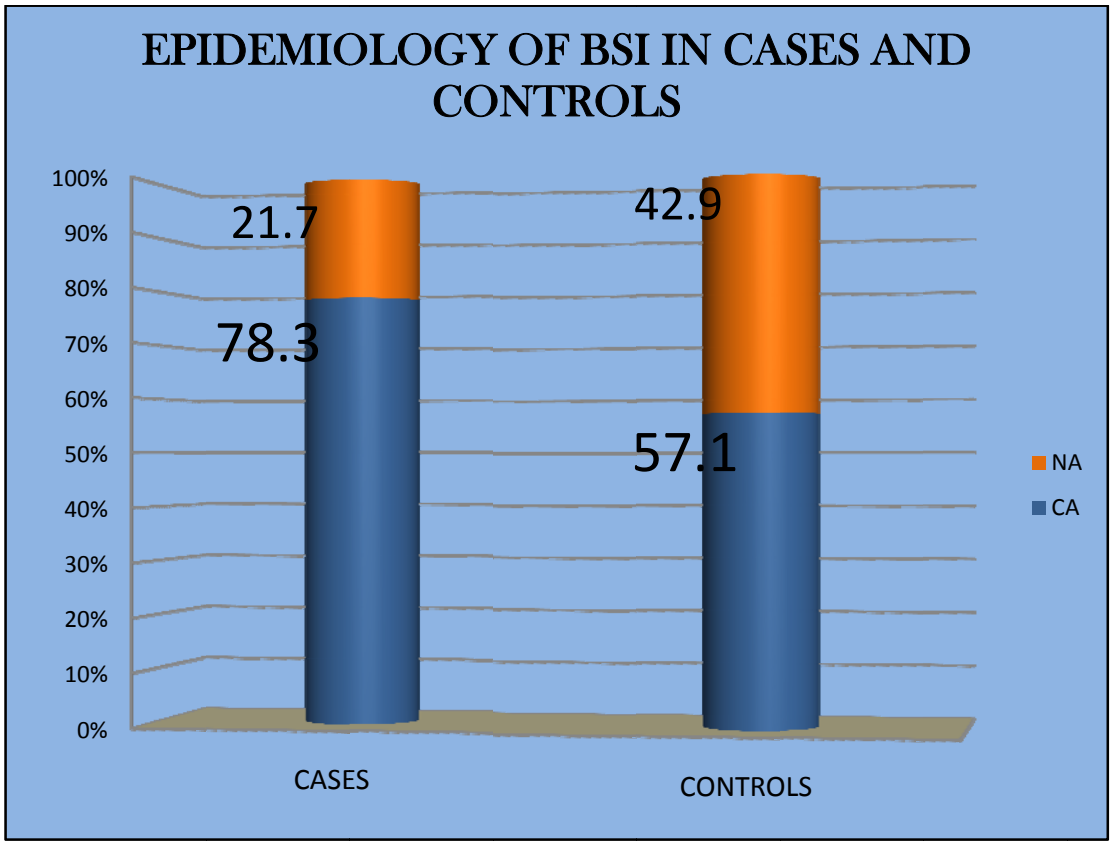
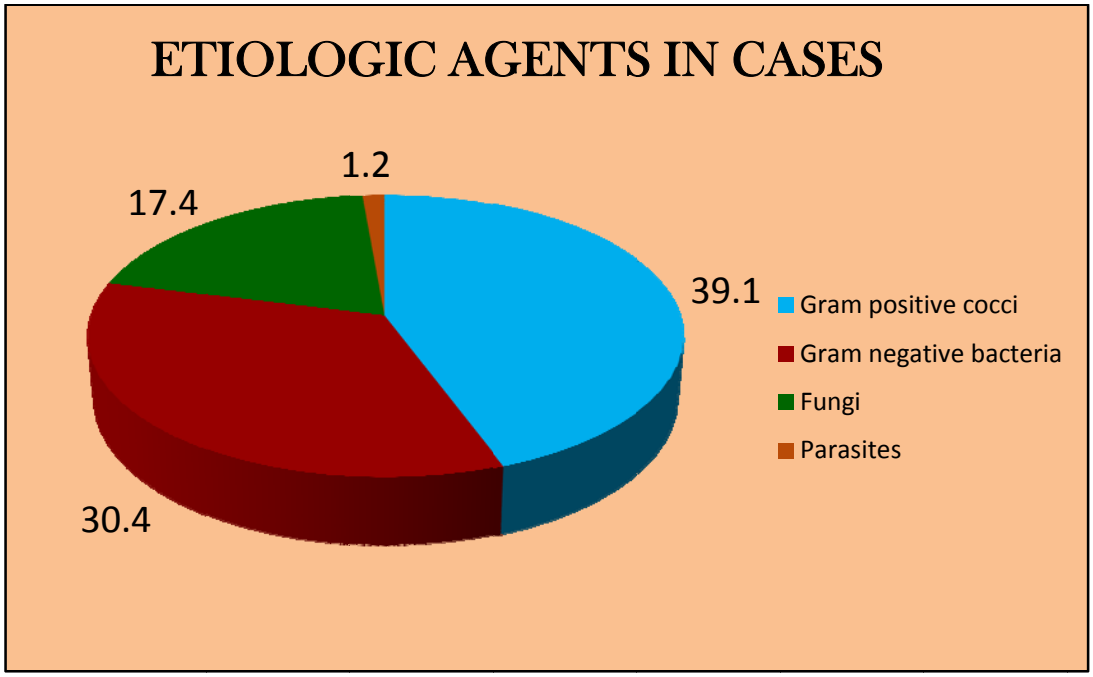

EPIDEMIOLOGY OF BSI IN CASES AND CONTROLS
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
21.7
78.3
42.9
57.1
CASES
CONTROLS
NA
CA

Table 13: ETIOLOGIC AGENTS IN THE STUDY POPULATION

Etiologic agents	Cases(n=23)		Controls (n=7)	
	No of Isolates	%	No of Isolates	%
GRAM POSITIVE COCCI	**9**	**39.1**	**4**	**57.1**
Staphylococcus epidermidis	4	17.4	2	28.5
Staphylococcus aureus	3	13	1	14.3
Staphylococcus schleiferi subsp schleiferi	1	4.3	-	-
Enterococcus fecalis	1	4.3	1	14.3
GRAM NEGATIVE BACTERIA	**7**	**30.4**	**3**	**42.8**
Escherichia coli	2	8.6	1	14.3
Proteus mirabilis	1	4.3	-	-
Proteus vulgaris	1	4.3	-	-
Citrobacter koseri	1	4.3	-	-
Klebsiella oxytoca	-	-	1	14.3
Salmonella paratyphi B	1	4.3	-	-
Pseudomonas aeruginosa	1	4.3	-	-
Acinetobacter spp	-	-	1	14.3
FUNGI	**4**	**17.4**	**-**	**-**
Candida albicans	2	8.6	-	-
Candida tropicalis	1	4.3	-	-
Cryptococcus spp	1	4.3	-	-
PARASITES	**3**	**13**	**-**	**-**
Plasmodium falciparum	1	4.3	-	-
Plasmodium vivax	2	8.6	-	-
Total	23	100	7	100

Gram positive bacteria were the commonest (39.1%).Coagulase negative Staphylococci was the most commonest isolate (21.7%) among cases.


ETIOLOGIC AGENTS IN CASES
1.2
17.4
39.1
30.4
Gram positive cocci
Gram negative bacteria
Fungi
Parasites

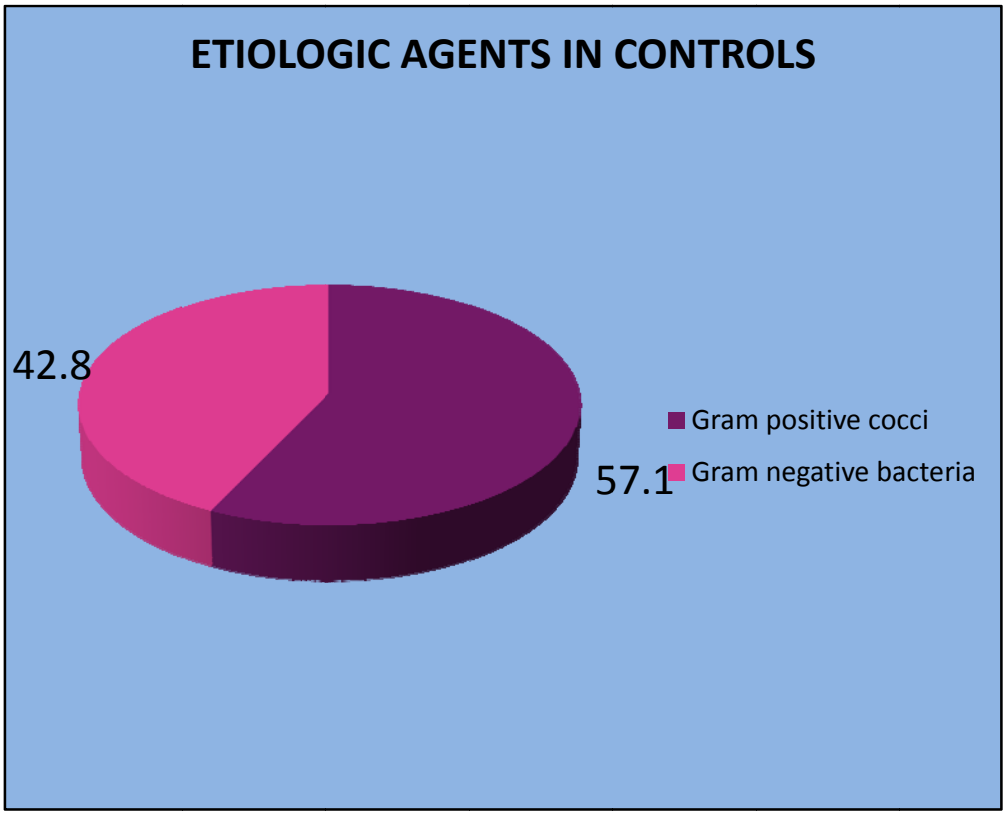

ETIOLOGIC AGENTS IN CONTROLS
42.8
57.1
Gram positive cocci
Gram negative bacteria

Table 14: ANTIMICROBIAL SUSCEPTIBILITY OF GRAM-POSITIVE BACTERIA CAUSING BSI IN HIV INFECTED INDIVIDUALS (CA-COMMUNITY ACQUIRED, NA-NOSOCOMIAL ACQUIRED)

SNO	Etiologic agents	Epidemiology of infection	Sensitivity for antibiotics								
			P	AC	Co	Ak	Cf	Cp	E	Ox	V
1	*Staphylococcus epidermidis*	CA	R	R	S	R	R	S	S	S	S
2	*Staphylococcus epidermidis*	NA	S	S	S	S	S	S	S	S	S
3	*Staphylococcus Epidermidis*	NA	R	R	S	S	S	S	R	S	S
4	*Staphylococcus epidermidis*	CA	R	S	R	R	S	R	S	S	S
5	*Staphylococcus aureus*	NA	R	S	S	S	R	S	R	S	S
6	*Staphylococcus aureus*	CA	R	R	S	S	S	R	S	S	S
7	*Staphylococcus aureus*	CA	S	S	R	R	S	S	S	S	S
8	*Staphylococcus schleiferi subsp schleiferi*	CA	R	S	R	R	S	S	S	S	S
9	*Enterococcus fecalis*	CA	R	S	-	R	S	-	S	R	S

Staphylococcus spp including *Staphylococcus aureus* were sensitive to many of the common antibiotics, specifically, none was resistant to methicillin and vancomycin. (P-Penicillin, AC-Amoxycillin Clavulanic acid, Co- Cotrimoxazole, E- Erythromycin, V-Vancomycin, Cp-Cephalexin, Cf-Ciprofloxacin, Ak-Amikacin, Ox- oxacillin)

Table 15: ANTIMICROBIAL SUSCEPTIBILITY OF GRAM-NEGATIVE BACTERIA CAUSING BSI IN HIV INFECTED INDIVIDUALS

SNO	Etiologic agents	Epidemiology of infection	Sensitivity for antibiotics								
			Cef	Cf	Of	Ak	Gm	CFS	C	TE	I
1	*Escherichia coli*	CA	S	S	S	R	R	S	S	S	S
2	*Escherichia coli*	CA	S	R	S	S	R	S	R	R	S
3	*Citrobacter koseri*	CA	S	S	S	S	S	S	S	-	S
4	*Salmonella paratyphi B*	CA	S	S	S	S	-	S	S	-	S
5	*Proteus vulgaris*	CA	S	S	R	S	R	S	R	R	S
6	*Proteus mirabilis*	CA	S	S	S	S	S	S	S	R	S
7	*Pseudomonas aeruginosa*	NA	S	S	S	S	R	S	S	R	S

All of the gram negative bacterias were sensitive to third generation of cephalosporins, indicating absence of ESBL production.

(Cef- Cefotaxime, Cf- Ciprofloxacin, Of- Ofloxacin, Ak- Amikacin, Gm- Gentamycin, CFS- Cefaperazone Sulbactum, TE-Tetracycline, C- Chloramphenicol, I- Imipenam)

Table 16: ANTIMICROBIAL SUSCEPTIBILITY OF GRAM-POSITIVE BACTERIA CAUSING BSI IN HIV UNINFECTED CONTROLS

SNO	Etiologic agents	Epidemiology of infection	Sensitivity for antibiotics								
			P	AC	Co	Ak	Cf	Cp	E	Ox	V
1	*Staphylococcus epidermidis*	CA	R	R	S	R	R	S	S	S	S
2	*Staphylococcus Epidermidis*	NA	R	R	R	R	S	R	S	R	S
3	*Staphylococcus aureus(MRSA)*	NA	R	R	R	R	S	R	R	R	S
4	*Enterococcus spp*	CA	R	R	R	R	S	R	S	-	S

Control group showed Methicillin resistance among nosocomial acquired strains of CoNS and *Staphylococcus aureus.*

Table 17: ANTIMICROBIAL SUSCEPTIBILITY OF GRAM-NEGATIVE BACTERIA CAUSING BSI IN HIV UNINFECTED CONTROLS

SNO	Etiologic agents	Epidemiology of infection	Sensitivity for antibiotics								
			Cef	Cf	Of	Ak	Gm	CFS	C	TE	I
1	*Escherichia coli*	CA	S	S	S	S	R	S	R	R	S
2	*Klebsiella oxytoca*	CA	R	R	S	R	R	R	R	R	S
3	*Acinetobacter spp*	NA	R	S	S	S	S	S	R	R	S

Klebsiella oxytoca was an ESBL producer, multidrug resistant, sensitive to ofloxacin and Imipenam.

Table 18: PREDISPOSING FACTORS OF BSI IN HIV INFECTED CASES

S no	Predisposing factors	Cases (n=100)				Controls(n=50)			
		With BSI		Without BSI		With BSI		Without BSI	
		No	%	No	%	No	%	No	%
1	Use of Intravenous catheter	7	30.4	12	15.6	3	42.8	2	4.7
2	Prev opportunistic Infections ≥2	10	43.5	21	27.27	-	-	-	-
3	Anemia	12	52.2	66	85.7	2	28.6	8	18.6
4	Neutropenia	8	34.8	3	3.9	1	14.3	-	-
5	Prev Antibiotic therapy	4	17.4	23	29.9	3	42.8	5	11.6

P=0.0003

In statistical analysis, by Pearson Chi- square test P=0.0003, intravenous catheters and anemia are independent factors associated with BSI in cases.

Table 19: SOURCE OF INFECTION IN BSI POSITIVE CASES

S no	Source of infection	Cases		Controls	
		No	%	No	%
1	Respiratory tract	1	4.3	1	14.3
2	IV catheter	5	21.7	2	28.6
3	Genitourinary tract	3	13	1	14.3
4	Skin	2	8.7	1	14.3
5	Bowel /Peritoneum	2	8.7	-	-
6	Biliary tract	0	0	-	-
7	Others	4	17.4	1	14.3
8	Unknown	8	34.8	1	14.3

Intravenous catheters (21.7%) were the common sources associated with BSI followed by genitourinary sources (13%).

Table 20: DISTRIBUTION OF PATHOGENS AMONG DIFFERENT AGE GROUPS AND GENDER IN STUDY POPULATION

Age (years)	Cases(n=100)						Controls(n=50)					
	Male			Female			Male			Female		
	Total	Positive	%	Total	Positive	%	Total	Positive	%	Total	Positive	%
16-20	3	1	4.34	1	-	-	4	-	-	3	1	14.3
21-30	16	2	8.7	10	1	4.34	4	-	-	7	1	14.3
31-40	24	7	30.4	11	2	8.7	7	1	14.3	6	-	-
41-50	18	7	30.4	4	1	4.34	3	-	-	3	-	-
51-60	7	2	8.7	3	-	-	3	3	42.8	1	1	14.3
>65	2	-	-	1	-	-	7	-	-	2	-	-
	70	19		30	4		28	4		22	3	

BSIs were common in age group of 31-40years (30.4%) and 41-50 years (30.4%) among male cases and 31-40 years (8.7%) of female cases, whereas BSI were common in 51-60 years (42.8%) of male control population.

Table -21:

BSI ISOLATES IN HIV POSITIVE INDIVIDUALS AND CD4 COUNTS

CD4 counts (cells/mm^3)	<100		100-200		200-500		Total
	Male	Female	Male	Female	Male	female	
Staphylococcus epidermidis	2	1	-	-	1	-	4
Staphylococcus aureus	1	-	1	1	-	-	3
Staphylococcus schleiferi subsp schleiferi	1	-	-	-	-	-	1
Enterococcus fecalis	1	-	-	-	-	-	1
Escherichia coli	-	-	1	-	1	-	2
Citrobacter koseri	-	-	1	-	-	-	1
Salmonella paratyphi B	1	-	-	-	-	-	1
Proteus vulgaris	-	-	1	-	-	-	1
Proteus mirabilis	-	-	-	-	-	1	1
Pseudomonas aeruginosa	-	-	1	-	-	-	1
Candida albicans	1	-	-	-	1	-	2
Candida tropicalis	1	-	-	-	-	-	1
Cryptococcus spp	1	-	-	-	-	-	1
Plasmodium falciparum	1	-	-	-	-	-	1
Plasmodium vivax	-	-	-	1	1	-	2
Total	10	1	5	2	4	1	23

Coagulase negative Staphylococci were commonly associated with BSIs in patients with CD4 counts <100 cells/m^3

Table 22: CORRELATION OF BSI WITH CD4 COUNTS (N=23)

CD4 range (cells/ uL of blood)	Cases (n=100)						
	Male (n=70)			Female (n=30)			Total positives %
	Total	Positive	%	Total	Positive	%	
<50	5	4	17.4	1	-	-	17.4
51-100	8	6	26.1	4	1	4.3	30.5
101-200	19	4	17.3	5	2	8.7	26.1
201-500	23	5	21.7	16	1	4.3	26.1
>500	15	-	-	4	-	-	-
	70	19		30	4		

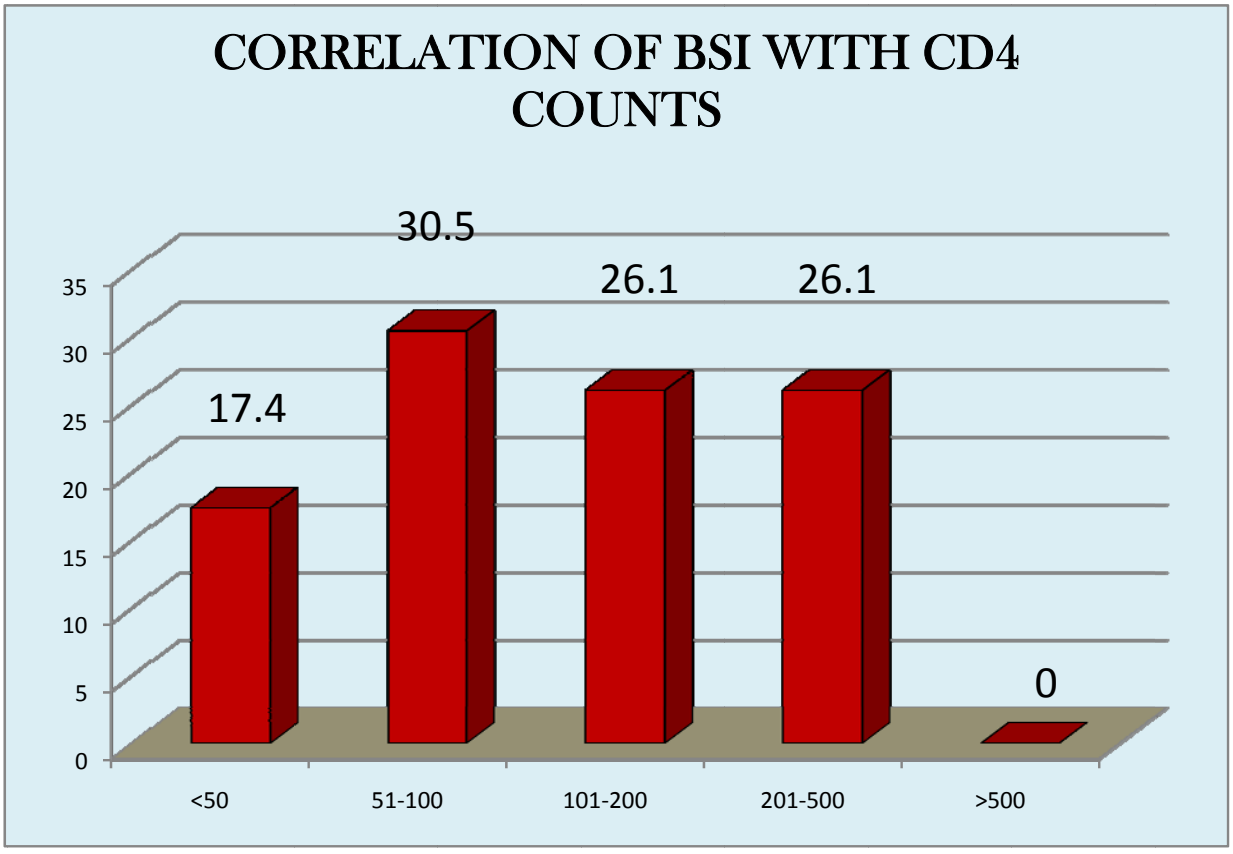
Most BSIs were common in CD4 counts less than 100(47.9%).

Table 23:

DISTRIBUTION OF CASES WITH REGARD TO HAART TREATMENT

	No of patients (n=100)	%	No of Patients with BSI	%
On HAART	72	72	18	78.3
Not on HAART	28	28	5	21.7
	100		23	

72% of the patients were under HAART and 28% of the patients were not on HAART. Majority of the patients were on HAART for duration below 3 months.


CORRELATION OF BSI WITH CD4 COUNTS
30.5
26.1
26.1
17.4
0
35
30
25
20
15
10
5
0
<50
51-100
101-200
201-500
>500

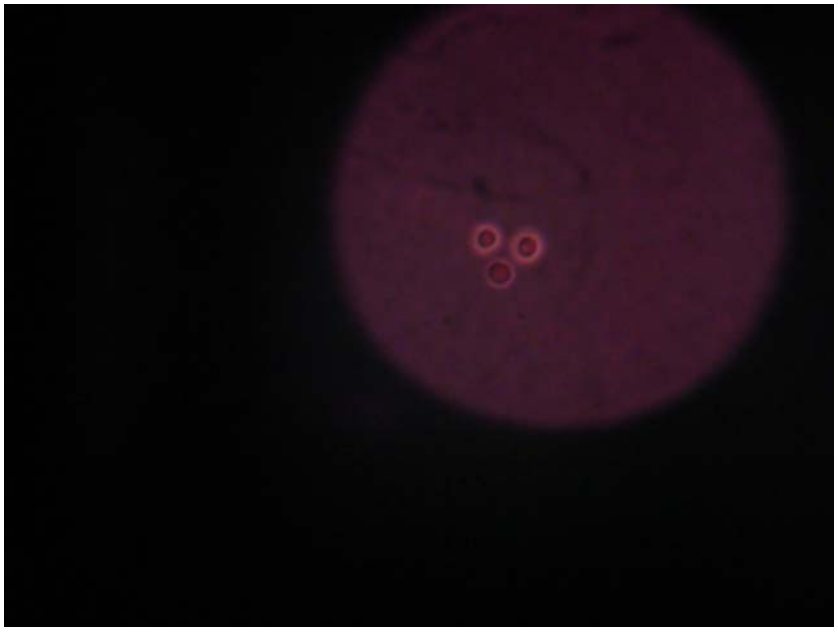
BRAIN HEART INFUSION BIPHASIC MEDIUM

TRYPTICASE SOY BROTH

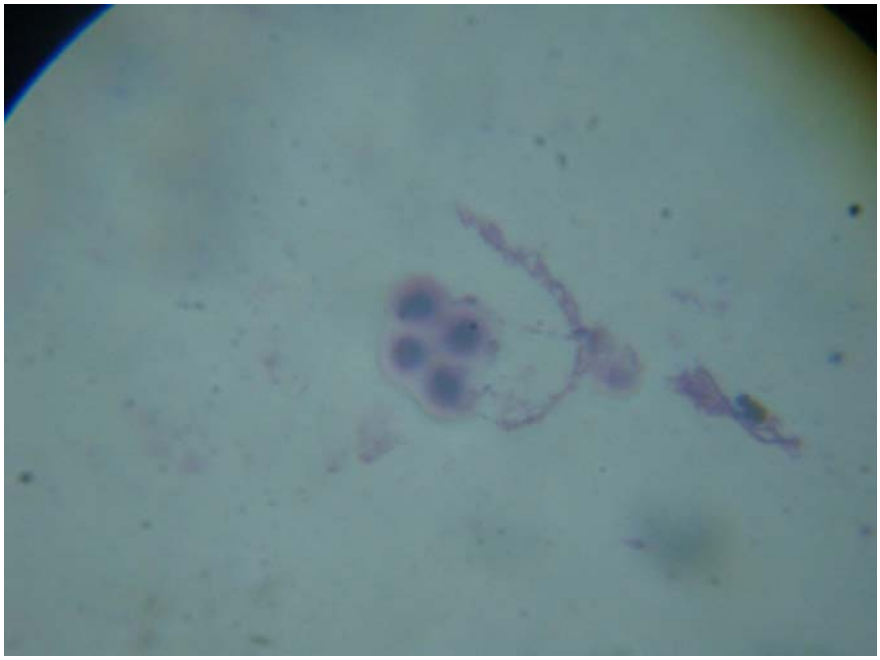
SDA SHOWING CYRPTOCOCCAL COLONIES

CRYTOCOCCAL SKIN GRANULOMAS

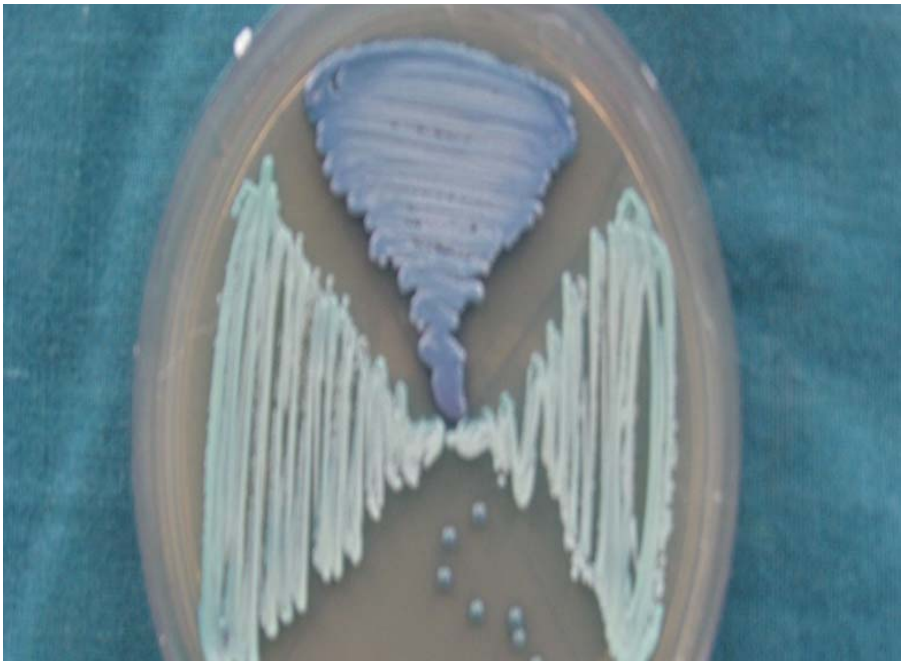
INDIA INK SHOWING YEAST CELLS WITH CAPSULE



GRAM STAIN- BUDDING YEAST CELLS WITH CAPSULE



SPECIATION OF CANDIDA SPP USING CHROM AGAR



BD FACS COUNTER

DISCUSSION

Bloodstream infections (BSIs) are a major cause of illness in HIV-infected persons. It has been noted that the pandemic affects mainly sexually-active age-groups, the highest prevalence was in patients with low CD4 counts and clinical signs of infection. Since the beginning of HIV disease, different studies have reported that bacterial infections were common in HIV infected patients because of abnormalities in humoral, cellular, and mucosal immunity. In addition, HIV-infected patients had an increased risk of bacteremia during bacterial infections [21, 73]. Hence this study was done to determine the prevalence of nonmycobacterial bacteremia and fungemia and determine the antimicrobial susceptibility pattern of the isolates from HIV patients and to correlate it with the CD4 count of the patients.

In the present study, majority of the patients were in the age group of 31-40 years (35%) (Table-3). This correlated with the study of CA Enwuru et al 2008, in which majority of the patients were within 31-40 years of age accounting for 42%. , and also the study of Omeñaca C and Turett G et al with 38% majority within 31-40 years of age. In contrast, in the study done by Bandar et al 2006, majority of the patients were in the age group of 20-30 years (85%).

There was a male preponderance accounting for 70% in this study (Table -3) VP Baradkar et 2009, Piroon Mootsikapun 2005, Prinscilla da et al 2002, also reported similar results in their studies. In contrast, Latiff et al 2004 showed female preponderance constituting 74.7% of the study group also Obi *et al*. established that there were more HIV-positive females than males in studies in South Africa, indicating a 'gender bias'[45].

Majority of the cases (66%) in our study population belonged to rural areas (Table-4) and most of their educational status (69%) were below higher secondary level (Table-5), depicting the lack of awareness regarding the disease prevention and early screening among the study population.

Table 6 shows high risk behaviours among the HIV infected cases. Homosexual behavior was prevalent among 8.5% of the study population, which was similar to two studies in high HIV prevalence areas of India, Mumbai and Chennai, prevalence rates of 9.6% and 6.8% were reported in MSM respectively by Godbole S et al and Mehendale S et al, 2005[24]. The pattern risk behavior showed large percentage of males (82.8%) had multiple sexual partners, similar to study of Kumar et al, 2008.

Majority of study population were in stage IV of disease (Table 7), done based on WHO staging for the year. CD4 counts of 39% of study cases were between 200- 500 (Table 8).

Comorbid conditions reported in this study were Cirrhosis (5%), renal diseases (11%), asthma and COPD (17%) were independent risk factors with diabetes mellitus (12%) and hypertension (22%) as per Table 9.

Fever was presenting symptom only in 30% of cases in the present study. The lower percentage presenting with fever may be attributed to the profound immunosuppression in these patients. Other neurological manifestations were seen in 18% and tubercular infection was found among 23% of study population. Weight loss (75%), and anemia (78%) were consistently associated with majority of cases as depicted in table 10.

In this study, blood stream infections were found to be 23 of 100 cases and 7 of 50 controls (Table11). Many studies by Fichtenbaum CJ et al 1995, Ssali F et al 1998, have demonstrated that the prevalence of bacteremia in HIV infected patients who have fever and are hospitalized, ranges from 5 to 28% . In 2000, Ippolito *et al*. also recorded 12.1% cases of bloodstream infections in a prospective multicentre study in HIV patients with advanced stage of the disease. Similarly, 30% prevalence rate of bloodstream infections was recorded in Malawian patients.

Epidemiology of infection among the cases and controls as in table 14 showed 21.7% infection were nosocomial acquired and 78.3 being community acquired. In HIV-infected patients, community-acquired and nosocomial bacteremia were found in 78.5%and 21.5%, respectively and

most were monomicrobial as revealed by Ssali et al,1998 in their study. This prevalence of bacteremia is consistent with earlier reports from similar patient populations in Rwanda and Kenya[55], but lower than the 42% prevalence reported in Tanzania[7]. In African studies in which both febrile and afebrile patients were included, the frequency of bacteremia was lower[52].

Etiologic agents were more commonly Gram positive bacteria (39.1%) with Coagulase negative Staphylococcus (17.4%), being the commonest isolate in both community acquired and nosocomial acquired cases (Table 15). Gram-positive bacteria are responsible for most of the bacteremias in hospitalized HIV-positive patients[62]. More Gram-positive than Gram-negative organisms were isolated from the blood stream of the subjects in a study by Chierakul et al, 2004. Gram negative bacteria isolated from the study subjects included *E.coli* (8.6%), *Proteus mirabilis* (4.3%), *Proteus vulgaris*(4.3%), *Salmonella paratyphiB* (4.3%), *Citrobacter koseri* (4.3%), *Pseudomonas aeruginosa* (4.3%).Fungal isolates like *Candida albicans* (4.3%), *Candida tropicalis* (4.3%), and *Cryptococcus neoformans* (4.3%). *Plasmodium vivax (8.6%)* and *Plasmodium falciparum(4.3%),* found among the isolates were identified by peripheral smear.

Non typhoidal Salmonella were commonly associated with blood stream infections as established earlier by the studies of Bonadio *et al*[9]. Many studies have shown Staphylococcus species to be the most common

isolates in blood[9, 20, 44]. Gooze and Choi *et al*. had established that coagulase-negative staphylococci are a major cause of infection in immuno-compromised patients[15,26.] Similarly, Bonadio *et al*. reported that the most frequently-isolated bacteria were coagulase-negative staphylococci[14].

Results of antimicrobial susceptibility tests in the present study, by Kirby Bauer Disk Diffusion method, revealed that most coagulase-negative staphylococci were susceptible to most antimicrobials screened. Specifically, none was resistant to methicillin and vancomycin (Table 15) compared with controls which were multidrug resistant (Table17). This is in line with results of previous studies, Shittu *et al*. 2006, reported that all isolates of *S. aureus* from clinical samples were susceptible to teicoplanin, vancomycin, fusidic acid, and rifampicin but showed high resistance to penicillin, sulphonamides, and tetracycline[56].

The Gram-negative bacterial isolates from this study also showed good sensitivity patterns to third generation of cephalosporins (Table 16) similar to the study with ceftazidin, cefotaxime, augmentin, and cefuroxime, by Adeyemi AI et al. 2010, indicating that they do not produce extended betalactamase . However, they too showed varying resistance to older-generation antibiotics, such as ampicillin, tetracycline, chloramphenicol. The multi-drug resistance patterns observed in this study are similar to those earlier reported by Adeleye and Adesoye[1].

The common sources of blood stream infection in patients with HIV infection include the lungs, skin, subcutaneous tissue, and intravascular catheters[41,49]. In the present study (Table 19), the most common source of BSI was intravascular catheters (21.7%), followed by genitourinary sources (13%), depicting intravascular catheter as a important predisposing factor causing bacteremia (Table 18), in correlation with study by Philips et al, 1995. No intravenous or other forms of drug abusers were observed in this study.

The present study also revealed, strong correlation of BSI with immunosuppression. Majority of patients (74%) with blood stream infection had CD4 counts less than 100 (Table 21 &22) similar to that observed in the study by Adeleye et al, where 67% of the patients had CD4 counts of <200 cells/μL of blood. In fact, 10% of outpatients with HIV and CD4 count <100 cells/mm3 had a BSI by Piroon Mootsikapun et al,2005, a prevalence similar to that seen in a previous study of febrile, HIV-infected inpatients in Thailand.

72% of study population was on HAART (Table 23). Most of the studies reported decreased incidence of BSIs in patients on ART. In this study, high prevalence of disease in patients with HAART (78.3%) may be because duration of HAART in these individuals were less than 3 months.

SUMMARY & CONCLUSION

Blood samples of randomly selected 100 HIV seropositive subjects inpatients (cases) and 50 seronegative inpatients (control), in Chennai, was examined for bloodstream infections. Blood culture in BHI biphasic medium and trypticase soy broth were done. Subcultures done in Blood agar, chocolate agar, differential agar and Sabouraud's dextrose agar in 48 hours. CD4 cell counts were done for HIV infected individuals and were used as indicators of immune status to analyze the results obtained.

- Blood stream infections were identified in 23% of cases and 7% of controls.
- Gram positive bacteria were more prevalent than gram negative bacteria.
- CoNS was the commonest pathogen identified in the cases (17.4%), followed by *Staphylococcus aureus* (13%), *Escherichia coli, Candida albicans, Plasmodium vivax* were 8.6% each.
- Other pathogens isolated include *Staphylococcus schleiferi subsp schleiferi, Enterococcus fecalis, Citrobacter koseri, Salmonella paratyphi B, Pseudomonas aeruginosa, Candida tropicalis, Cryptococcus neoformans,* and *Plasmodium falciparum.*
- Among controls, CoNS was the commonest isolate but showed multidrug resistance.
- CoNS, isolated from repeated subcultures alone were considered significant, others were taken as insignificant and reported as no growth.

- Methicillin resistance and ESBL producers were not found among the isolates. The spectrum of pathogens causing BSI in these HIV patients were mainly the normal microflora of the local environment showing sensitivity to most antimicrobials.
- Intravenous catheters constituted as a significant predisposing factor.
- BSI were common in age group of 31-50years (60.8%) of male cases and 31-40 years (8.7%) of female cases, whereas BSI were common in 51-60 years (42.8%) of male control population.
- Majority of cases with blood stream infections were found to have CD4 cell counts <100cells/mm^3.
- Duration of HAART also influences the incidence and mortality , due to BSI.

BSI in adult HIV-infected patients is often caused by Gram-positive pathogens in both Community Acquired and Nosocomial settings. Coagulase negative Staphylococci are increasingly associated with blood stream infections in these patients, especially in profound immunosuppression with CD4 counts <100 cells/mm^3.

Early detection of HIV infected individuals, timely review of these patients with their CD4 counts, thereby leading to initiation of HAART at the earliest will prevent bacteremia, fungemia, and other opportunistic infections, decreasing the mortality due to these illnesses.

PROFORMA

1. Name :
2. Patient Identity No/ART No. :
3. Age/ Sex :
4. Marital status :
5. Address :
6. Education :
7. Occupation :
8. Religion :
9. Referring Centre (if any) :

II. RISK FACTOR QUESTIONNAIRE

1. Reasons for HIV testing
   (Voluntary/Antenatal Check Up/Referral from STI clinic/
   Positive Consort/Symptomatic conditions attributing to
   HIV infection/AIDS defining Illness/Others) :
2. H/o Smoking/ Alcohol intake/ Drug abuse :
3. H/o Blood Transfusion/ any previous surgery/
   Dental procedures :
4. H/o tattooing :
5. H/o needle stick injuries in
   health care professionals :

6. H/o Spouse HIV+ve/ Parent to Child transmission:
7. H/o Premarital Sex/ Exposure to multiple sexual partners/

   Exposed to CSWs :
8. H/o use of contraception :
   a. Type
9. H/o homosexual partner :
10. H/o comorbid conditions :

    (Diabetes/Hypertension/Coronary artery disease)

II. ILLNESS DEFINING HISTORY

1. H/o fever
   a. Duration
   b. Treatment (if any)
2. H/o weight loss
3. H/o persistent diarrhea
   a. Duration
   b. Treatment (if any)
4. H/o generalized/localized lymphadenopathy
5. H/o persistent cough
   a. Duration
   b. Treatment (if any)
6. H/o tubercular infection
   a. Treatment (if any)
7. H/o genital ulcers/vesicles
8. H/o VDRL tested reactive

9. H/o Vaginal Discharge
    a. If any, Nature of discharge
    b. Blood stained/not
10. H/o PAP smear/biopsy/Post coital bleed
11. H/o visual Changes/blindness
12. H/o headache
    a. Nature
    b. Duration
    c. Associated with nausea/vomiting
13. H/o neurological symptoms/
    Neurologic signs on referral card
        a. Memory deficits
        b. Numbness
        c. Weakness in limbs
        d. Seizures
14. H/o Skin lesions
15. H/o mood disorders
16. H/o date of initiation of ART
17. H/o treatment for OIs
18. H/o of drug adverse effects

Laboratory Evaluation:

- Hematological Investigation:
    1. Hemoglobin
    2. Complete blood count
- Others: CXR

- Microbiological Investigation

HIV testing

CD4 counts

Peripheral smear by Leishman's staining

Blood cultures in

Brain Heart Infusion Biphasic medium

Trypticase soy broth

Subcultures in

Blood Agar

Chocolate Agar

Nutrient Agar

MacConkey Agar

Biochemical reactions

Antimicrobial susceptibility testing by Disc Diffusion method

ABBREIVATIONS

HIV- Human Immunodeficiency Virus

AIDS- Acquired Immuno Deficiency Syndrome

CD-Cluster Differentiation

BSI- Blood Stream Infections

CMV- Cytomegalovirus

ART- Anti Retroviral Therapy

HAART- Highly Active Anti Retroviral Therapy

NACO- National AIDS Control Organisation

APPENDIX

I. STAINING

Gram staining

Methyl violet (2%) – l0g Methyl violet in l00ml absolute alcohol in 1litre of distilled water (primary stain)

Grams Iodine – l0g Iodine in 20g KI (fixative)

Acetone – Decolourising agent

Carbol fuchsin 1% – Secondary stain

India Ink Staining

India Ink 150 ml

Merthiolate (1:1000) 3 ml

Tween 80 (1:10,000) 0.1ml

Mixed, filtered and kept in bottles.

Leishman staining

0.15% Leishman powder dissolved in 100% methanol. Used after 24hrs.

Phosphate buffer (Sorensen)

Stock A: 0.2M sodium di-hydrogen orthophosphate (mw 156).

3.12g dissolved in 100ml distilled water.

Stock B: 0.2M di-sodium hydrogen orthophosphate (mw 142).

2.83g dissolved in 100ml ditilled water.

25.5ml of A added to 24.5ml of B, pH adjusted to 6.8

II. CULTURE MEDIA

Brain Heart Infusion Biphasic Medium

Sodium citrate lgm

Sodium chloride 4gm

Sodium phosphate 5gm

Dextrose l0 gm

Peptone 1 0gm

Brain Heart Infusion Broth

Brain infusion broth 250ml

Heart infusion broth 750ml

Sodium polyanethol sulphonate 0.25gm

Obtain Ox brain and heart. Remove all fat from the heart. Cut into small pieces and grind. Add distilled water three times and keep at 4°C overnight. From the brain, remove meninges fully and then, weigh. Add distilled water and mash by using hand. Keep in the cooler over night. Next morning boil the brain and heart separately for 30 minutes. Then filtered through cotton layer. Measure each broth separately. Mix both infusions and

the remaining ingredients. Dissolve well and adjust pH of the entire amount to 7.4 to 7.6. Autoclave at 121° C for 15 minutes.

Brain Heart Infusion Agar Slant

Same as above, in addition it contains agar. Autoclave at 121°C for 15 minutes. Distribute in screw capped bottles and slant is allowed to solidify, then broth is distributed in bottles.

Trypticase Soy broth

Pancreatic digest or casein	17.0 gram
Enzymatic Soy Digest	3.0 gram
Sodium chloride	5.0 gram
Dipotassium phosphate	2.5 gram
Dextrose	2.5 gram
Sodium Polyanetholsulfonate	0.3gram

Final pH adjusted to 7.3± 0.2. Autoclave at 121°C for 15 minutes and distribute in screw capped bottles .

Mac Conkey agar

Peptone	-	20g
Sodium taurocholate	-	5g
Distilled Water	-	1 ltr
Agar	-	20g
2% neutral red in 50% ethanol	-	3.5ml
10% lactose solution	-	l00mI

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix. Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15 minutes.

Blood agar (5% sheep blood agar)

Peptone	-	l0g
Nacl	-	5g
Distilled water	-	1 Ltr
Agar	-	10g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood (sterile) at 55°C adjust pH to 7.4.

Chocolate Agar

Sterile defibrinated blood	-	10 ml
Nutrient Agar (melted)	-	100 ml

When the temperature was about 75°C, sterile blood was added with constant agitation. After addition of blood, kept in water bath and heating was continued till the blood changed to chocolate colour. Cooled to about 50° C and poured about 15ml into petri dishes with sterile precaution.

Sabouraud's Dextrose Agar With Antibiotics

Composition of Sabouraud's Dextrose Agar (Emmons Modification)

Dextrose	-	20 gm
Peptone	-	10 g
Agar	-	20 g
Distilled water	-	1000 ml
Final pH	-	6.9

The ingredients are dissolved by boiling. Gentamycin was dissolved in 10 ml of 95% alcohol and added to boiling medium. Autoclave at 121°C for 15 minutes, dispense in tubes and allow to cool in slanted position.

Mueller- Hinton Agar

Beef infusion	-	300ml
Caesein hydrolysate	-	17.5g
Starch	-	1.5g
Agar	-	10g
Distilled water	-	1ltr
pH	=	7.4

Sterilise by autoclaving at 1210C for 20 mins

Cornmeal Agar

Cornmeal	-	40g

Agar - 15g

Water - 1 litre

Boil the cornmeal in 1 litre of water for 60 min.Filter through muslin and add the agar. Steam to dissolve, dispense in required amounts and autoclave at 115° C for 30min. allow to cool to 50°C and pour approximately 20 ml amounts into Petri dishes.

II. MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION OF BACTERIA

Oxidase Reagent

Tetra methyl p-phenelene diamine dihyrochloride-

1% aqueous solution.

Catalase

3% hydrogen peroxide

Indole test

Kovac's reagent

Amyl or isoamyl alcohol - 150ml

Para dimethyl amino benzaldehyde - 10g

Concentrated hydrochloric acid - 50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

Christensen's Urease test medium

Peptone	-	1g
Sodium chloride	-	5g
Dipotassium hydrogen phosphate	-	2g
Phenol red	-	6ml
Agar	-	20g
Distilled water	-	1 ltr
10% sterile solution of glucose	-	l0ml
Sterile 20% urea solution	-	l00ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121°C for 30min. Cool to about 50°C, add the glucose & urea, and tube the medium as slopes.

Simmon's Citrate Medium

Koser's medium	-	1 ltr
Agar	-	20g
Bromothymol blue 0.2%	-	40ml

Dispense, autoclave' at 121°C for 15 min and allow to set as slopes

Triple Sugar Iron medium

Beef Extract	-	3g
Yeast extract	-	3g

Peptone	-	20g
Glucose	-	1g
Lactose	-	10g
Sucrose	-	10g
Ferric citrate	-	0.3g
Sodium chloride	-	5g
Sodium thiosulphate	-	0.3g
Agar	-	12g
Phenol red 0.2% solution	-	12ml
Distilled water	-	1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

Glucose phosphate broth

Peptone	-	5g
Dipotassium hydrogen phosphate	-	5g
Water	-	1 ltr
Glucose 10% solution	-	50ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filter dispense in 5ml amounts and sterilize at 121°c for 15min. Sterilize the glucose solution by filtration and add 0.25ml to each tube.

Methyl Red Reagent

Methyl Red - 10mg

Ethyl alcohol - 30ml

Distilled water - 20ml

Voges Proskauer Reagent

Reagent A: Alpha naphthol - 5g

Ethyl alcohol - 100ml

Reagent B: Potassium hydroxide - 40g

Distilled water - 100ml

Peptone water fermentation test medium.

To the basal medium of peptone water, add sterilised sugars of 1% indicator bromothymol blue with Durham's tube.

Basal medium - peptone water

Sugar solutions:

Sugar - 1ml

Distilled water - l00ml

pH = 7.6.

Mannitol motility medium

Agar - 5g

Peptone - 1g

Potassium nitrate - 1g

Mannitol - 2g

Phenol red indicator

Distilled water - l000ml

pH = 7.2

Potassium Nitrate Broth

Potassium nitrate (KN03) - 0.2gm

Peptone - 5.0gm

Distilled water - 100ml

The above ingredients were mixed and transferred into tubes in 5 ml amount and autoclaved.

Phenyl Alanine Deaminase Test

Yeast Extract 3g

Dl-Phenylalamine 2 g

Disodium hydrogen phosphate lg

Sodium Chloride - 5 g

Agar 12g

Distilled water - 1 lr

pH adjusted to 7.4, distributed in tubes and sterilized by autoclaving at 121° C for 1 5 minutes, allowed to solidify as long slopes.

ganisation

OI- Opportunistic Infections

ESBL- Extended Spectrum Beta Lactamase

MRSA- Methicillin Resistant *Staphylococcus aureus*

SPS- Sodium polyanethol sulfonate

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